RAPID COMMUNICATION

Clonality in Juvenile Chronic Myelogenous Leukemia

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Juvenile chronic myelogenous leukemia (JCML) is a myeloproliferative disease in which morbidity and mortality are primarily caused by nonhematopoietic organ failure from myelomonocytic infiltration or by failure of the normal bone marrow. Morphologic evidence of maturation arrest, karyotypic abnormalities, and progression to blast crisis are frequent events. Viral infections and other reactive processes can initially mimic the clinical course of JCML, creating diagnostic problems. Because of the rarity of JCML and technical limitations, formal clonality studies have not been reported previously. Nine female JCML patients were identified by clinical criteria, characteristic 'spontaneous' in vitro cell growth, and negative cultures and titers for various viral agents. Peripheral blood and bone marrow samples were obtained at the time of diagnosis for cell separation and RNA and DNA isolation. To assess clonality, X-chromosome inactivation patterns were evaluated using three different, recently developed polymerase chain reaction-based clonality assays. All nine female JCML patients showed evidence for monoclonal origin of mononuclear cells at the time of diagnosis. Cell separation studies further traced the monoclonal origin back to at least the most primitive myeloid progenitor cell. Reversion to a polyclonal state was demonstrated after bone marrow transplant and also in one patient following treatment with 13-cis retinoic acid. This demonstration of clonality in JCML delineates it from the reactive processes and provides a basis for molecular genetic strategies to identify causally associated mutations.

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of this polymorphism in only a single racial group has limited its usefulness. Second-generation clonality assays examine for restriction fragment X-chromosome DNA genomic polymorphisms and distinguish active from inactive X-linked alleles on the basis of differences in methylation patterns at the polymorphic locus. X-chromosome DNA probes available for methylation analysis include the phosphoglycerate kinase (PGK) gene, the hypoxanthine phosphoribosyl transferase (HPRT) gene, the human androgen receptor gene (HUMARA), and the hypervariable DXS255 locus (M27β). The major limitation of methylation-based clonality assays is the potential for incomplete (unreliable) methylation patterns of some DNA sequences. The PGK gene and HPRT genes have relatively reliable methylation patterns but are limited because of modest informativeness (<35%) in the general female population. On the other hand, the M27β probe is highly informative but has less reliable methylation patterns. The HUMARA gene is less well studied but has high informativeness and appears to have reliable methylation patterns. More recently, expression-based HUMARA assays have shown good correlation between methylation patterns and gene expression at this locus. A third type of clonality assessment is based on the detection of a transcriptional polymorphism of the active X chromosome. There is a C T polymorphism that occurs at the exonic nucleotide no. 1311 of the G6PD gene. This technique uses a polymerase chain reaction (PCR)-based gene expression analysis with reverse transcription of G6PD mRNA, followed by the ligase chain/detection reaction for analysis of the polymorphism. This assay has the advantages of not depending on methylation patterns and allowing the study of non-nucleated cells such as reticulocytes and platelets, but has the disadvantage of only modest informativeness at the G6PD C T locus.

In this report, our approach using multiple clonality assays obviated the above-described limitations in each assay, and showed evidence of clonality in JCMl at the time of diagnosis.

MATERIALS AND METHODS

Peripheral blood (PB) and BM samples from untreated, newly diagnosed female JCMl patients were used in this study. Diagnostic criteria for JCMl included leukocytosis with monocytosis, hepatosplenomegaly, absence of chromosomal abnormalities, less than 25% marrow blasts, negative viral titers and cultures, and in vitro studies showing 'spontaneous' colony growth and selective hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF) as described previously. Patients with monosomy 7 were excluded. With parental consent and the approval of the respective Institutional Review Boards, PB and/or BM aspirate samples were collected into preservative-free heparin (100 U/mL) and shipped at ambient room temperature overnight for analysis. Some of the studies described below were performed at two institutions, with some differences in methods. Both sets of results were compared for confirmation.

Cell Separation and RNA and DNA Isolation

Neutrophils (PMN) cells were separated from mononuclear cells (MNC) by density gradient centrifugation on Ficoll-Paque (Pharmacia, Uppsala, Sweden) or Histopaque-1077 (Sigma, St Louis, MO) depending on the institution. PMNs were isolated in the erythrocytic phase (>95% purity by Wright stain) after hypotonic lysis of the red blood cells (RBCs). PMNs were subsequently lysed with Triton X-100 (Sigma) lysis solution (0.32 mol/L sucrose; 10 mmol/L TRIS pH 7.5; 5 mmol/L MgCl2; 1% Triton X-100). Nonadherent cells in the MNC were isolated from adherent cells by different methods. In the first, performed at the University of Alabama at Birmingham (UAB), after Histopaque separation MNC were washed three times with Hanks' balanced salt solution (HBSS) (GIBCO, Grand Island, NY) and then plated in HBSS with 13% human AB serum. After three successive adherence depletion steps as previously described, nonadherent cells (progenitor and lymphocyte-rich) were harvested and lysed in Triton X-100 buffer. Adherent cells (monocytes) were isolated using a rigorous adherence, washing, and scraping protocol as described previously and lysed with Triton X-100. In the second method, performed at the Brigham and Women's Hospital, MNC isolated from Ficoll-Paque were washed three times with Iscove's Modified Dulbecco's Medium (IMDM)/2% fetal calf serum (FCS) (GIBCO) and plated on Lux dishes (Nunc Inc, Naperville, IL) in IMDM/10% FCS at 37°C overnight in a humidified incubator with 5% CO2. Nonadherent cells were procured and lysed in Triton X-100. Adherent cells were recovered by scraping the Lux dishes with subsequent lysis in Triton X-100. Platelets were isolated by differential centrifugation to obtain platelet-rich plasma, then pelleted and lysed with Triton X-100.

RNA Isolation and Preparation

Total reticulocyte RNA was isolated by selectively lysing peripheral blood erythrocytes, then isolating RNA as described by Goosens and Kun with the modification of an additional purification of the acid RNA precipitate by the guanidine HCl-phenol method. The RNA from the other cells studied was prepared by using the guanidine HCl-phenol method. The RNA aliquots were used for preparations of cDNA template by reverse PCR reaction from the total cellular RNA by random priming.

DNA Isolation and Preparation

All isolated cell populations were digested with SDS (5%/protease K (2 mg/mL) buffer at 37°C for 24 hours, then the DNA was extracted with phenol, phenol/chloroform and chloroform, precipitated with 2 vol of absolute ethanol and 1/10 vol of 3 mol/L sodium acetate, and resuspended in either TRIS-EDTA (TE) buffer or ultrapure H2O. The isolation of DNA from cells obtained from fluorescence-activated cell sorting (FACS) requires modification of the above protocol because of the small number of cells. Flow sorted cells are transferred to a 2-mL microfuge tube, centrifuged at 14,000 rpm in a microfuge for 5 minutes, decanted, resuspended in 270 μL of digestion buffer (75 mmol/L NaCl, 25 mmol/L EDTA) and 30 μL of SDS/protease K solution, incubated for 4 hours at 50°C, then extracted with phenol/chloroform and chloroform. One microliter of glycogen (Boehringer Mannheim, Indianapolis, IN) is added followed by 30 μL of 3 mol/L sodium acetate, and 2 vol of absolute ethanol is added for precipitation. Tubes are immersed in liquid nitrogen for 2 minutes then thawed while centrifuged at 14,000 rpm for 30 minutes. Tubes are carefully decanted and the pellet is washed with 70% ethanol. DNA is resuspended in 10 to 20 μL of H2O according to the number of cells at the starting procedure. Ten microliters of the solution is used for the HpaII restriction endonuclease digestion step.

Flow Sorted Cells Analysis

Lineage Depletion

To remove mature cells from BM samples and to enrich for hematopoietic progenitors, we performed negative selection as described previously. Briefly, nonadherent cells were concentrated by centrifugation and resuspended in 1 μL FCS/106 cells. Antibodies against
surface markers on mature hematopoietic cells were added. Murine monoclonal antibodies (MoAbs) against CD2, CD5, CD11b, CD15, CD19, CD14 (all from Becton Dickinson, Mountain View, CA), and anti-glycophorin A (Dr W. Bigbee, University of Pittsburgh, PA) were used. The BM was incubated on ice for 30 minutes and washed three times with IMDM/2% FCS. Immunomagnetic beads (Dynabeads M-450; Dynal Inc, Great Neck, NY) coated with sheep-anti-mouse IgG were added to cells and incubated for 30 minutes. Lineage positive cells were removed with a Dynal magnetic particle concentrator MPC-1 (Dynal Inc). The lineage negative fraction (approximately 1.5% to 2% of nonadherent cells) was incubated with a combination of a phycoerythrin (PE)-conjugated antibody against CD38 (Becton Dickinson) and a fluorescein isothiocyanate (FITC)-conjugated antibody against CD34 (AMAC Inc, Westbrook, ME). The cells were washed and resuspended in a small volume of PBS/2% BSA for sorting.

FACS. Immunofluorescence analysis and cell sorting was performed on a FACStar Plus cell sorter (Becton Dickinson) with a Hewlett Packard computer system (Palo Alto, CA). FITC and PE fluorescence were excited using an Innovia 90 argon laser (Coherent, Palo Alto, CA) tuned at a wavelength of 488 nm with 200 mW power output. For analysis and sorting, the Consort 32 operating system (Hewlett Packard) and LYSIS II software (Becton Dickinson) was used. Sort windows were drawn on the CD34 (FITC') and CD38 (PE') BM population. Lineage positive cells were also analyzed and sorted using a window for CD14+ cells and the CD14- BM population.

Clonality Assays Based on Differential DNA Methylation of X Chromosomes

HUMARA Clonality Assay

The HUMARA assay was recently developed23 and was performed with minor modifications and is explained in detail in a recent publication.28

Quantitation of alleles. Dried gels were exposed to a phosphor screen for 24 hours, then scanned on a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The ratio between the two X-linked alleles was measured using the ImageQuant software (Molecular Dynamics). The allele ratio is the ratio between the two X-linked alleles in a given sample. The corrected ratio (Cr) is the allele ratio of the digested sample divided by the allele ratio of the nondigested sample of the same specimen. This ratio compensates for potential preferential amplification of the one active allele.

PGK-PCR Clonality Assay (PPCA)

This technique has been described in detail elsewhere.27,28 As a further confirmation of findings, this assay was performed by both groups in this report with minor differences in technique as described below. In the PPCA performed at the Brigham and Women’s Hospital, genomic DNA was initially digested with HpaII as described for the HUMARA clonality assay with the omission of the RsaI enzyme. A nondigested sample was used as an auto-control.

PCR amplification of the PGK locus. A PCR mix containing 8 µL of 10X buffer (same as above), 8 µL of dNTP (200 µmol/L each), 4 µL (20 pmol) each of primer 2A and 2B,0.2 µL (1 U) of Taq polymerase (Cetus, Norwalk, CT), and 52 µL of H2O was added to the microfuge tube used for the digestion. Tubes were overlaid with 50 µL of mineral oil.

Amplification parameters. Denaturation occurred at 94°C for 3 minutes, then 50 cycles of PCR with the following parameters: 1 minute at 94°C; 1 minute 45 seconds at 58°C; 2 minutes 45 seconds at 72°C. A nested set of primers (Research Genetics, Huntsville, AL) exactly as described previously27 except that 40 cycles were used for each amplification instead of 60 cycles.

Polyclonal samples have both the 530- and 433-bp bands with a ratio between the two alleles close to 3:1 because of heteroduplexing between the BstXI-positive strand and the BstXI-negative strand during PCR. These heteroduplexes do not digest with BstXI and artificially increase the relative proportion of the 530-bp allele. A sample is judged to be polyclonal if both alleles are visualized, whereas a clonal sample will either have only one of the two alleles present, or will demonstrate a major shift in the 3:1 ratio after BstXI digestion. There was good concordance of results and conclusions in the PGK-PCR assays performed at the two institutions.

Clonality Assay Based on the Detection of a Transcriptional Polymorphism on the Active X Chromosome

G6PD C T Polymorphism Clonality Assay

These analyses use detection of an active X chromosome transcriptional polymorphism by amplification of the polymorphic region of the G6PD gene by PCR, and then detection of the polymorphism by the ligase detection reaction (LDR) using thermostable ligase that covalently binds with adjacent DNA oligonucleotides, provided they are perfectly complementary to target DNA in the region of ligation.29,30,39 LDR analysis was performed either using a genomic DNA or a cDNA template prepared by reverse transcription-PCR reaction from total cellular RNA.35 The LDR assay was performed as described,29,30 with the following modifications. The target consists of 10 ng of DNA and NAD+ at 1 mmol/L. A cloned thermostable ligase,40 a kind gift from Dr F. Barany (New York, NY), was diluted in 10 mmol/L Tris HCl pH 8.0 in 50% glycerol containing 0.5 mmol/L EDTA, 1 mmol/L dithiothreitol, 100 µg bovine serum albumin (Fraction V), and 0.1% Triton X-100. The PCR primers for amplification of the genomic DNA are as previously published.29,30 Thirty cycles of ligation reaction are used at 1 minute at 94°C and 4 minutes at 65°C. The reaction products are separated on a 10% polyacrylamide gel containing 7 mol/L urea and detected by autoradiography. For more details justifying the design and principles of LDR, refer to the original method description.29,30,39 Evidence for clonality is based on the loss of either the T allele (a 46-bp nucleotide fusion product) or the C allele (a 44-bp nucleotide fusion product).

RESULTS

Table 1 depicts demographic information for the nine JCM patients used in this study, as well as the clonality assays used for each patient. The patients are numbered according to the JCML registration system at UAB. One of the nine female JCM patients, J22, proved informative for a transcriptional polymorphism at the C T locus in the exonic nucleotide no. 1311 of the G6PD gene. Using the ligase chain/detection reaction, cDNA templates prepared from total cellular RNA were analyzed from isolated populations of mononuclear cells, granulocytes, monocytes, platelets, and reticulocytes from the patient. The results (Fig 1)
show that all of these separate cell populations were clonally derived at the time of diagnosis in patient J22 because all cells expressed only the C allele (44-bp product). This patient was subsequently treated with 13-cis retinoic acid but unfortunately had progressive disease and succumbed.

Of the other eight JCML patients, clonality information was obtainable by the HUMARA assay for patients J9, J10, J15, J35, and J39, whereas the PGK-PCR assay provided information on patients J9, J10, J15, J20, J31, J35, J39, and J40 (Table 1). All of these tests showed monoclonal derivation of cells at the time of diagnosis in all of these patients. Figure 2 shows a composite HUMARA assay demonstrating clonality in four representative JCML patient mononuclear samples at the time of diagnosis, before initiation of any therapy. The process of amplification of short tandem repeats gives rise to a phenomenon known as shadow banding, supposedly caused by slippage of the Taq polymerase; thus, the presence of 3 to 4 bands in the HUMARA assay. The ratio analysis in the HUMARA assay, as analyzed on the PhosphorImager, showed that greater than 95% of cells were clonally derived. Therefore, very few normal hematopoietic elements remained at the time of diagnosis. Figure 3 shows a composite PGK-PCR assay for three other representative JCML patients at the time of diagnosis. Because both the HUMARA and the PGK-PCR assays are also PCR-based, they are, like the ligase chain/detection reaction, sensitive assays for clonality assessment from minute cell populations, and in addition are highly informative.

One patient, J35, was analyzed for clonality at several timepoints through the course of her disease (Fig 4), both by HUMARA and by PGK. After diagnosis and initial assessment, she was treated with 13-cis retinoic acid for several months according to Pediatric Oncology Group protocol no. 9265.

Concurrent with a partial response to retinoic acid, there was evidence for some reversion to a polyclonal state (Figs 5 and 6). However, because of increasing leukocytosis,
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Patient J9 | Patient J10 | Patient J35 | Patient J39
---|---|---|---
- | + | - | +
- | - | + | -
- | - | - | +
- | - | - | -

Fig 2. HUMARA analysis32 of genomic DNA isolated from PB mononuclear cells from four female JCML patients at the time of diagnosis. DNA samples were analyzed using a PCR assay as described with the human androgen-receptor locus. Lanes +, PCR after HpaII predigestion; lanes -, PCR without predigestion. Clonality is shown by the presence of only the active X allele after digestion (ie, presence of lower set of bands but loss of upper bands in + lanes). The process of amplification of short tandem repeats gives rise to a phenomenon known as shadow banding, supposedly caused by slippage of the Taq polymerase. Thus, the presence of 3 to 4 bands in the HUMARA assay.

Retinoic acid was discontinued and she received an allogeneic BM transplant (BMT). Unfortunately, she failed to engraft (data not shown) and within months had clinical re-emergence of JCML, concomitant with a recrudescence of clonal hematopoiesis (Fig 5). At this timepoint, cells isolated by lineage depletion and flow sorting showed that both CD14+ and CD14- fractions were clonal (Fig 7). Further cell separation and isolation demonstrated that early stem and progenitor cells, marked by CD34+, CD38- (Fig 8, region 2) were also clonal by HUMARA analysis. This provided excellent correlation with the results in patient J22 (Fig 1) from the ligase chain/detection reaction, showing that all cell populations derived from a cell as primitive as at least the myeloid stem cell level were clonal. Although these data imply that lymphocytes are also clonal, indicating that JCML is a pluripotent stem cell disorder, definitive lymphocyte separation and clonality assessment are yet to be performed on future samples. Two other patients in this study, J9 and J15, had clinical responses to retinoic acid. However, the data in these two cases were inconclusive in regard to a significant shift toward polyclonality after retinoic acid therapy (data not shown). Patient J15 has subsequently received an allogeneic BMT. Since the transplant she has no evidence of disease and demonstrates a polyclonal pattern by PGK-PCR assay, in contrast to the clonal pattern at diagnosis (data not shown).

In all of the above clonality assays, we attempted to use somatic controls to address extreme skewing of Lyonization1 when possible. Many of the samples were previously stored specimens and thus other tissue not available. Patient J35 had skin tissue available and did not show skewing in the HUMARA assay (Fig 6) or in the PGK-PCR assay (Fig 5). Further, the incidence of excessive skewing appears

Patient J15 | Patient J20 | Patient J20 | Patient J40
---|---|---|---
+ | - | + | -
- | + | - | -
- | - | - | +
- | - | - | -

Fig 3. PGK-PCR analysis33 of genomic DNA isolated from peripheral blood mononuclear cells (MNC) or purified neutrophils (PMN) from three female JCML patients at the time of diagnosis. Lanes +, PCR after HpaII predigestion; lanes -, PCR without predigestion. A sample is judged to be polyclonal if both alleles are visualized, whereas a clonal sample will either have only one of the two alleles present, or will show a major shift from the baseline 3:1 ratio after BstX1 cutting.
lower in children. Excessive skewing to the degree necessary to give the results in Fig 1 has not been observed in normals with the LCR/LDR assay. Finally, the ultimate control to rule out this phenomenon was observed in JCML patient J35 when a reversion from clonality to polyclonality was observed.

DISCUSSION

JCML is an interesting disorder that occurs in early childhood. Most patients are ages 3 to 24 months at diagnosis, though it is seen in patients up to 4 years of age. It is rarely seen in the neonatal period and thus not considered a congenital disease. The disease is typically more fulminant than most other chronic myeloproliferative disorders with a median survival of less than 10 months. JCML can pose a diagnostic dilemma for pediatricians. Early phase JCML can clinically resemble infection from Epstein-Barr virus, cytomegalovirus, or other agents, and it can be difficult to distinguish from other myeloproliferative syndromes because the myelomonocytic proliferation is morphologically normal, as is the karyotype. There is a male predilection of approximately 2:1, similar to the pattern in myeloproliferative syndrome with monosomy 7, but the diseases appear to be distinct. Because JCML phenotypically resembles certain histiocytic syndromes, one could envision that the disease is confined to the monocytic lineage. Therefore, these clinical aspects of JCML, especially similarity to other diseases, raise the question as to whether this is a clonal versus a polyclonal process, especially because clonality analysis in other myeloproliferative disorders has yielded variable results.

The somatic mutation theory of carcinogenesis posits that tumors are the result of a monoclonal expansion of a single cell with selective growth advantage because of acquired somatic mutation(s). Thus, the assessment of the clonal derivation of cells is an essential element of the characterization and definition of a malignancy. The assays available presently for clonality assessment all suffer from limitations. The most widely used assays depend on differential methylation patterns between the active and inactive X-chromosomes at various polymorphic loci. Limitations may arise due to either low levels of informativeness at the particular locus or due to unreliable differential methylation patterns. Of the available probes, the HUMARA gene is highly informative and appears to have reliable differential methylation. A different, recently developed, clonality assay uses the transcriptional analysis of the active X chromosome based on a DNA exonic polymorphism detected by the ligase chain reaction and circumvents the methylation limitation. But the polymorphic locus presently used for this assay is limited by modest informativeness. Other exonic polymorphic loci with higher levels of heterozygosity are being tested currently. Therefore, if one wants to examine the clonal nature of a particular disorder from limited numbers of female patients, the most logical approach may be to use several of the assays to corroborate results. This was the approach used in the present study to prove the clonal nature of hematopoiesis at the time of diagnosis in JCML.

Our results indicate that the hematopoietic mononuclear progenitor and stem cells are clonal at the time of diagnosis in 9/9 of the JCML patients tested. Furthermore, we show in one patient that CD34+, CD38 early progenitor cells are clonally derived when examined by the HUMARA assay, as are the reticulocytes, platelets, monocytes, and granulocytes in a second patient examined by the ligase chain/detection reaction. These data definitively show that JCML is not a pure monocytic disorder. Rather, it is the result of a clone arising from the earliest myeloid stem/progenitor cell, or possibly a more primitive stem cell. It could potentially arise from different levels of early progenitors in different patients, as noted in some cases of essential thrombocythemia. Furthermore, it remains unclear whether the mutation(s) causing JCML can confer self-renewing potential on the myeloid stem cell, a feature not thought to be present at this level in normal hematopoiesis.

Another intriguing preliminary finding from the clonality assays in this study is the partial reversion to polyclonality demonstrated in one patient, following a partial clinical response to 13-cis retinoic acid (CRA) therapy. JCML patients' response to chemotherapy is generally poor, and BMT appears to be curative in some, but is hindered by a high relapse rate. CRA therapy has shown some promise in a pilot trial and is under further evaluation in a formal phase II protocol (Pediatric Oncology Group no. 9265). Patient J35 in this study demonstrated re-emergence of polyclonal hematopoiesis with CRA therapy, whereas the evidence in two other patients was inconclusive (data not shown). This phenomenon is being further investigated through the phase II protocol. Interestingly, this potential switch or modulation with CRA parallels other changes observed in JCML cell-growth patterns in vitro.

In vitro, JCML cells show characteristic exuberant 'spontaneous' growth of colony forming units-granulocyte/macrophage (CFU-GM) in the absence of exogenous growth factors or stimuli at very low cell densities. Yet JCML is not an autocrine-driven disease because the 'spontaneous'
Fig 5. PGK-PCR analysis\(^{37}\) of genomic DNA isolated from PB mononuclear cells from JCML patient, J35, successively through her disease course. Lanes +, PCR after \textit{Hpall} predigestion; lanes − , PCR without predigestion. Skin biopsy sample shows polyclonal derivation of nonhematopoietic tissues. Because of size considerations, the lanes without predigestion (−) have been deleted after the initial sample. The re-emergence of the 433-bp allele indicates a temporary reversion to a partial polyclonal state associated with therapies as indicated in Fig 4.

Fig 6. HUMARA analysis\(^{39}\) of genomic DNA isolated from various cell populations as indicated from JCML patient, J35, during the polyclonal phase of her disease as indicated in Figs 4 and 5. All samples in this figure show polyclonality. DNA samples analyzed using PCR assay as described with the human androgen-receptor locus. Control, PCR without predigestion; \textit{Hpall}− , PCR after \textit{Hpall} predigestion.

growth of the unfractionated, nonadherent hematopoietic progenitor cells is dependent on the presence of monocytes. Removal of monocytes, and potentially other cells, by adherence to plastic before culture of the cells abrogates the 'spontaneous' growth.\(^{32}\) However, it is not a truly paracrine-driven disease either, because excessive cytokine production by JCML monocytes is inconsistent.\(^{32}\) Rather, the consistent finding is one of selective hypersensitivity by the nonadherent progenitors to GM-CSF, but not to interleukin-3 (IL-3) or granulocyte-CSF (G-CSF).\(^{32}\) Other cytokines produced
by the monocytes have been implicated in JCML, and may play an indirect role by stimulating GM-CSF production in monocytes (IL-1, tumor necrosis factor-α [TNFα] or may suppress normal hematopoiesis (TNFα). However, the direct and central role of selective hypersensitivity to GM-CSF points to an aberration in the GM-CSF signal transduction pathway. To date, binding studies and sequence analysis of the GM-CSF receptor have shown no abnormalities (K.S.Z. and P.D.E., unpublished observations, August 1994), in contrast to mutations identified in cytokine receptors in some congenital hematopoietic diseases. Recent reports have shown allelic loss of the NFI gene in some NF-1 patients with JCML, and N-ras mutations in other JCML patients. Both NFI loss and N-ras mutations may activate the Ras signal transduction pathway. GM-CSF is also known to activate Ras-GTP, an intriguing potential connection considering the GM-CSF hypersensitivity of JCML cells. Yet, RAS mutations are observed in a broad spectrum of neoplasms. Thus, determining whether RAS mutations are involved in the pathogenesis of JCML will require further study.

In summary, JCML is a clonal disorder at the time of...
diagnosis evolving from an abnormal myeloid stem cell, or possibly an earlier level. But is it a malignant process from the start? The Knudson “two-hit” model of malignancy proposes that the first “hit” gives a proliferative advantage to a clonally derived population that may be phenotypically and karyotypically normal, such as JCMCL at diagnosis. The second “hit” then leads to the malignant phenotype. One could postulate that early phase JCMCL is the consequence of a single mutation conferring a proliferative/survival advantage. But because of marked monocytosis, JCMCL patients suffer earlier and greater morbidity than in other myeloproliferative disorders because excessive monocyte numbers create more problems than do excessive RBCs (ie, polycythemia vera) or platelets (ie, essential thrombocytosis). If so, then why is JCMCL so much more difficult to control with chemotherapy than these disorders? The answers to these and other questions may come from molecular genetic strategies aimed at identifying mutations responsible for the aberrancies in the signal transduction pathways leading to cytokine hypersensitivity, such as GM-CSF in JCMCL, and insulin-like growth factor-1 (IGF-1) hypersensitivity in polycythemia vera.

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