We have compared the proliferative and cytotoxic capacities of a highly purified population of recombinant interleukin-2 (rIL-2)-activated peripheral blood mononuclear cells (PBMCN), termed adherent lymphokine-activated killer cells (A-LAK), in 15 chronic phase (CP) and 10 advanced disease (AD) Ph-positive chronic myelogenous leukemia (CML) patients. The selective enrichment of CML A-LAK cells depended on their propensity to adhere to plastic and to proliferate when cultured in the presence of rIL-2 for 14 days. In both CP and AD patients, 14-day culture resulted in growth of a uniform population of large granular lymphocytes. While less than 10% of the A-LAK cells were CD56−/CD3+ (mature T lymphocytes), 82% ± 12% of A-LAK cells from early CP patients (diagnosed less than 1 year from study), 84% ± 3% of A-LAK cells from late CP patients (studied greater than 1 year after diagnosis), and 87% ± 3% of A-LAK cells from AD patients were CD56+/CD3− (activated natural killer [NK] cells). No bcr gene rearrangement could be found in A-LAK cells from 15 CP and six AD CML patients studied. A-LAK cells from seven early CP CML patients displayed similar cytotoxicity against K562 (80% ± 7% lysis at effector:target ratio of 20:1) and against Raji (80% ± 12% lysis) compared with A-LAK from 17 normal individuals (72% ± 3% K562 lysis, P = .21; 74% ± 5% Raji lysis, P = .39). However, the cytotoxicity of A-LAK cells from eight late CP patients (59% ± 5% K562 lysis, P = .02; 52% ± 8% Raji lysis, P = .02) and that of 10 AD patients studied at any point after diagnosis (31% ± 3% K562 lysis, P < .001; 25% ± 8% Raji lysis, P < .001) was significantly lower than that of seven early CP CML patients and 17 normals. The proliferative potential of A-LAK cells from seven early CP CML patients (291 ± 191-fold) was significantly greater than that of A-LAK cells from 17 normal individuals (23 ± 3-fold, P = .03), eight late CP patients (46 ± 17-fold, P = .02), and 10 AD patients (5.4 ± 1.9-fold, P = .01). In contrast to CML A-LAK, K562 cytotoxicity of unstimulated mature peripheral blood NK cells was significantly lower in early CP CML patients than in normals and remained low at all stages of disease. A population of benign, highly cytotoxic, hyperproliferative IL-2 sensitive mononuclear cells termed A-LAK can be generated from the peripheral blood of early CP CML patients. Either prolonged duration of CP disease or occurrence of advanced disease is associated with significant diminution of both cytotoxic and proliferative capacities of the A-LAK population that cannot be explained by malignant transformation of the A-LAK cells. Furthermore, the disparity in early CP CML patients between the markedly diminished K562 cytotoxicity found in the circulating unstimulated, mature NK cells compared with that of A-LAK cells suggests that the A-LAK culture method reveals a population of NK progenitors whose cytotoxic potential is not accurately reflected in conventional cytotoxicity assays of mature peripheral blood NK cells. Exploration of the defects in cytotoxicity of killer cells obtained after A-LAK culture may illuminate mechanisms underlying escape from control associated with disease progression in CML and other leukemias.

CHRONIC MYELOGENOUS leukemia (CML) is a malignant disorder of the hematopoietic stem cell characterized by the Philadelphia chromosome (Ph) and gene rearrangement in the breakpoint cluster region on chromosome 22 (bcr). Without major therapeutic intervention, such as allogeneic bone marrow transplantation, the disease usually evolves from a stable chronic phase (CP) to blast crisis after 3 to 4 years. Blast crisis is often preceded by an accelerated phase during which the disease becomes refractory to standard therapy, and additional clonal evolution may occur. This transformation from chronic to advanced phase suggests that the disease may gradually escape from control mechanisms still actively present during the early period after diagnosis.

Natural killer (NK) cells play an important role in tumor control. Previous studies suggest that NK cell activity in peripheral blood of CML patients is significantly decreased; however, isolation of purified populations of NK cells has been difficult in CML because the peripheral blood is flooded with malignant myeloid progenitors. In vitro culture of peripheral blood mononuclear cells (PBMC) from patients with CML results in growth of lymphokine-activated killer cells, or LAK cells. The LAK population is heterogeneous, consisting of highly cytotoxic activated NK cells and a large fraction of less cytotoxic T cells. Using a recently described “adherence” method, we have demonstrated that a relatively pure population of recombinant interleukin-2 (rIL-2)-stimulated cells, termed adherent lymphokine-activated killer cells (A-LAK), can be generated from the blood of patients with CP CML. The A-LAK population consists of benign, highly cytotoxic cells with morphologic and phenotypic characteristics of activated NK cells.

We hypothesize that a progressive inability of NK cell populations to respond adequately to the presence of malignant cells may contribute to the escape from control associated with transformation to accelerated phase and blast crisis.

Diminished A-LAK Cytotoxicity and Proliferation Accompany Disease Progression in Chronic Myelogenous Leukemia

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in CML. To explore this hypothesis we examined the function and characteristics of the A-LAK population generated from chronic phase and advanced phase CML patients.

PATIENTS AND METHODS

**Patient population.** Fifteen Ph-positive CP CML patients, 10 Ph-positive advanced disease (AD) patients, and 17 normal individuals were studied. Seven CP patients were studied within 1 year after diagnosis (early CP), and eight were studied from 19 months to 6 years after diagnosis (late CP). Three of 10 AD patients were studied within 1 year after diagnosis (7/10 < 1 year after diagnosis). Of the 10 AD patients, four patients were in blast crises at the time of study, and one patient was in third chronic phase. Two patients had extensive reticular bone marrow fibrosis, one patient had additional cytogenetic abnormalities, two patients had persistent thrombocytopenia unresponsive to conventional therapy (hydroxyurea/busulfan), and one patient had increasing splenomegaly. The white blood cell (WBC) count at time of study was comparable in seven early CP (24.7 ± 6.2 x 10^9/L), eight late CP (17.9 ± 3.6 x 10^9/L), and 10 AD (17.5 ± 4.5 x 10^9/L) patients. Of 15 CP patients, three had never received therapy before study. Five of 15 CP and 6 of 10 AD patients received hydroxyurea only, 7 of 15 CP patients received hydroxyurea and busulphan, and 4 of 10 AD patients had been treated with combination chemotherapy. All therapy was stopped at least 5 days before study. All patient and control samples were obtained using guidelines approved by the Committee on the Use of Human Subjects at the University of Minnesota, Minneapolis, MN.

**Generation of A-LAK cells.** A-LAK cells were generated as described previously. PBMCs were depleted of monocytes in a 2-hour plastic adherence. Monocyte-depleted PBMCs (2 x 10^6/mL when PB WBC count was greater than 20 x 10^9/L) were then incubated in culture medium consisting of RPMI 1640 with 10% human heat inactivated AB serum (Pel-Freeze Biologicals, Brown Deer, WI), 2 mmol/L L-glutamine (GIBCO, Grand Island, NY), 2 x 10^-5 mol/L 2-mercaptoethanol, antibiotics (penicillin, 1,000 U/mL and streptomycin, 100 U/mL; GIBCO), and 1,000 IU rIL-2 (Cetus, Emeryville, CA) at 37°C and 5% CO2 for 24 hours. After 24 hours, the nonadherent fraction was decanted, the culture flasks were washed vigorously with warm RPMI, and the adherent fraction was cultured for up to 14 days in complete medium supplemented with 1,000 IU rIL-2. In contrast to the originally described method, we omitted addition of autologous A-LAK conditioned media at 24 hours of culture to prevent inadvertent addition of soluble suppressive factors derived from the malignant myeloid precursor cells. The cell concentration was adjusted to 1 to 2 x 10^6/mL at each feeding every 36 to 48 hours. Unstimulated NK cells were obtained by Ficoll-Hypaque separation and subsequent 2-hour plastic adherence to deplete monocytes.

**Cytotoxicity assays.** Day 14 A-LAK cells were tested for cytotoxicity against an NK sensitive K562 cell line and an NK resistant Raji cell line in a 4-hour CR release assay. The cytotoxic capacity of unstimulated NK cells was tested against K562 only. Effector:target ratios ranged from 20:1 to 0.66:1. Results are given at an effector:target ratio of 20:1. All determinations were done in triplicate, and percentage lysis was determined using the following equation:

Experimental Mean cpm - Spontaneous Release Mean cpm 
Total Release Mean cpm - Spontaneous Release Mean cpm 

**Proliferation.** We obtained an index of A-LAK cell proliferation by calculating the total number of A-LAK cells obtained after 14 days of culture divided by the absolute number of CD16^+ / CD3^- cells (determined by two-color fluorescence-activated cell sorter [FACS] analysis) present in the cell population used to initiate the cultures at day 0. This method was used since the cells recovered after 14 days of culture are not derived from the total number of cells plated at day 0, but from the adherent A-LAK precursor cell fraction retained after decanting the nonadherent population at 24 hours of culture.

**Phenotype.** Cell surface antigens were determined by direct staining of the cells with monoclonal antibodies (Becton-Dickinson, Mountain View, CA). Antibodies used were directed at CD3 (Leu-4), CD16 (Leu-11a), CD56 (NKH1/Leu-19). Phenotypic analyses were done with a FACS IV flow cytometer (Becton-Dickinson) equipped with a consort 40 computer. Fluorescein and phycoerythrin-coupled mouse isotype-matched immunoglobulins were used to control for nonspecific labeling.

**Hematopoietic progenitor assay.** A-LAK cells were plated in triplicate at a final concentration of 2.5 x 10^9/mL in methylcellulose (final concentration 1.12%) with Iacove's modified Dulbecco's medium (IMDM). Culture medium was supplemented with 30% fetal calf serum (GIBCO), 5% phytohemagglutin-Leukocyte conditioned medium (PHA-LCM), 3 U human urinary erythropoietin (Terry Fox Laboratories, Vancouver, British Columbia) and 10^-3 mol/L 2-mercaptoethanol. Cultures were incubated in four-well plates (Nunclene, Naperville, IL) at 37°C in a humidified atmosphere containing 5% CO2 for 14 days then assessed for evidence of burst-forming units-erythroid (BFU-E) or mixed lineage colony-forming unit (CFU-Mix) formation as described.

**Detection of bcr gene rearrangement.** High molecular weight DNA was extracted from myeloid cells and day-14 A-LAK cells using the nuclei extraction technique. Gene rearrangement studies used the gel transfer technique described by Southern, with transfer of Bgl II-digested DNA to a nylon membrane. A 1.95 Bgl II/HindIII genomic DNA probe for the bcr containing Exon 1 of this region was a gift from Dr David Leibowitz. This probe was radiolabeled by nick translation, and the filter was hybridized according to the manufacturer's recommendations. After hybridization, the filter was washed (final wash in 0.1 x standard sodium citrate [SSC], 0.5% sodium dodecyl sulfate [SDS] at 65°C, for 60 minutes) and exposed to X-ray film for 24 hours at -70°C.

**Statistical analysis.** Results of experimental points obtained from multiple experiments were reported as the mean ± 1 standard error of the mean (SEM). Significance levels were determined by two-sided Students t test analysis.

RESULTS

**Characteristics of day-14 A-LAK cells.** Morphologic examination of Wright-Giemsa–stained day-14 A-LAK cells from 17 normals, 15 CP CML patients, and 10 AD CML patients revealed that this cell population consists uniquely of large cells with the morphology of large granular lymphocytes (Fig 1). Phenotypic analysis of day-14 A-LAK cells showed that the enrichment for activated NK cells (CD56^+ / CD3^-) is similar in seven early CP (82% ± 12%), eight late CP (84% ± 3%), 10 AD (87% ± 3%) patients, and 17 normals (78% ± 5%; Fig 2). The contamination with T cells (CD56^- /CD3^+) was less than 10% in A-LAK populations from either CP patients, AD patients, or normals. However, A-LAK cells from the different CML subgroups could be distinguished on the basis of quantitative expression of the CD56 surface antigen (CD56^high^ versus CD56^dim^). A-LAK cells derived from the peripheral blood of seven early CP patients contained significantly greater numbers of CD56^high^ cells (CD56^high^, 85% ± 4%; CD56^dim^, 14% ± 2.2%) than day-14 A-LAK cells from eight late CP patients (CD56^high^, 40% ± 5%; CD56^dim^, 60% ± 5%;
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Fig 1. Wright-Giemsa stained cytospin preparations of day-14 A-LAK cells generated from the peripheral blood of one early CP (A) and one AD CML patient (B).

P < .001) and 10 AD patients (CD56bright+, 32% ± 8%; CD56dim+, 67% ± 7.8%; P < .001) (Fig 3).

Day-14 A-LAK cells generated from the peripheral blood of all normal individuals, 12 CP patients, and 10 AD CML patients were cultured under conditions that promote the growth of myeloid progenitors. None of these cultures revealed growth of CFU-C, BFU-E, or CFU-MIX, suggesting that the A-LAK cell population is not contaminated with viable hematopoietic progenitors.

Southern blot analysis of day-14 A-LAK cells failed to reveal the presence of the bcr gene rearrangement in A-LAK cells from 13 CP and six AD CML patients known to have a bcr gene rearrangement in myeloid cells (Fig 4).

Cytotoxicity of day-14 A-LAK cells. The cytotoxicity of A-LAK cells from seven early CP CML patients against an NK-sensitive target K562 (80% ± 4% lysis at effector:target ratio of 20:1) or an NK-resistant target Raji (80% ± 12% lysis) was similar to that of normals (72% ± 3% K562 lysis, P = .2; 74% ± 5% Raji lysis, P = .39). However, the lytic capacity of A-LAK cells from eight late CP patients against K562 (59% ± 5% lysis) and Raji (52% ± 8% lysis) was significantly lower than that of normals (P = .02 and P = .03, respectively) and seven early CP CML patients (P = .02 and P = .02, respectively). The cytotoxicity of A-LAK cells from 10 AD CML patients at any point after diagnosis (31% ± 3% lysis of K562 targets and 25% ± 6% lysis of Raji targets) was also significantly lower than that of normal individuals (P = .001 and P = .01, respectively) and early CP CML patients (P < .001 and P < .001, respectively) (Fig 5).

In order to exclude the possibility that prolonged or more extensive treatment underlies the differences in cytotoxic activity shown between early CP, late CP, and AD patients, we compared the cytotoxicity of A-LAK cells from three untreated and four treated early CP CML patients. We were unable to demonstrate a significant difference between untreated and treated patients, either in K562 lysis (77% ± 5% lysis versus 80% ± 5% lysis; P = .9) or Raji lysis (83% ± 7% lysis versus 74% ± 7% lysis; P = .4) (Fig 6). We were also unable to demonstrate differences in cytotoxic capacity of A-LAK cells derived from AD patients treated with hydroxyurea alone (n = 6) or in combination with other agents (n = 4) against either K562 targets (34% ± 6% lysis versus 33% ± 7% lysis; P = .8) or Raji targets (26% ± 9% lysis versus 23% ± 2% lysis; P = .9) (Fig 7). Three of four patients with CML in blast crisis had received therapy with...
**Fig 3.** Representative single color histogram (y axis, relative number of cells; x axis, fluorescence) displaying CD56\(^{+}\) and CD58\(^{+}\) A-LAK cell subpopulations in early CP CML (A), late CP CML (B), and AD CML (C) patients. Significantly greater numbers of CD56\(^{+}\) A-LAK cells are CD56\(^{+}\) in A-LAK cells generated in an early CP CML patient (A: CD56\(^{+}\), 79.2%) than in a late CP patient (B: CD56\(^{+}\), 63.5%) and an AD CML patient (C: CD56\(^{+}\), 24.5%). In each histogram, the experimental histogram (solid line) is superimposed over the PE IgG1 control histogram (dotted line).

hydroxyurea only and were studied 1, 3, and 6 months after diagnosis. The A-LAK proliferation and cytotoxicity in these relatively untreated patients with CML in blast crisis were significantly diminished in comparison with seven early CP patients, demonstrating that the diminished cytotoxicity is associated with disease phase rather than extent or duration of therapy.

**Proliferative potential of day-14 A-LAK cells.** Since the A-LAK cells recovered after 14 days of culture are not derived from the total number of cells plated at day 0, but from the adherent cell fraction retained after decanting the nonadherent population on day 1 of culture, we calculated an index of proliferation by dividing the total number of A-LAK cells obtained at day 14 of culture by the absolute number of CD16\(^{+}/\)CD3\(^{-}\) cells (determined by two-color FACS analysis) present in the cell population used to initiate the cultures at day 0:14 PBMC from patients with CML obtained after density gradient separation and 2-hour plastic adherence remain heavily contaminated with immature myeloid precursor cells. Since these immature myeloid precursor cells adhere to plastic when incubated for 24 hours, we were unable to calculate the A-LAK proliferation as the total number of cells obtained after 14 days of culture divided by the total number of cells adherent to the plastic after 24 hours, as originally described by Medler et al.\(^{13}\) The 291 ± 191-fold expansion in A-LAK cell cultures from early CP CML patients is significantly greater than that in A-LAK cultures from normal individuals (23 ± 3-fold; \(P = .02\)), late CP patients (38 ± 15-fold; \(P = .03\)), and 10 AD CML patients (5.4 ± 1.9-fold; \(P = .01\)) (Fig 8). Proliferation of A-LAK cells from eight late CP patients is similar to that of 17 normals (\(P = .16\)), while the expansion of A-LAK cells from 10 AD CML patients studied at any point is significantly lower than that of 17 normal individuals (\(P < .001\)) and eight late CP patients (\(P = .01\)). We were unable to show a significant correlation between proliferation of A-LAK cells with either the percent CD16\(^{+}/\)CD3\(^{-}\) cells present in the PBMC used to initiate the cultures, the PB WBC count, or the absolute number of circulating CD16\(^{+}/\)CD3\(^{-}\) cells. Comparison of A-LAK proliferation between untreated and treated early CP patients and between AD patients treated with either hydroxyurea only or in combination with other agents did not show significant differences.

**Cytotoxicity of unstimulated, mature NK cells.** We analyzed the cytotoxicity of unstimulated mature NK cells from the peripheral blood of CP and AD CML patients and normal individuals. Since the percent of CD16\(^{+}/\)CD3\(^{-}\) cells present in the partially purified NK population derived from CP (3% ± 2.99%; \(P = .085\)) and AD (2.7% ± 2.1%; \(P = .054\)) patients was significantly lower than that in normals (6.9% ± 2.9%), we selected 18 normal individuals (CD16\(^{+}/\)CD3\(^{-}\) fraction, 4.5% ± 1%), 13 CP CML patients (4.8% ± 1%), and eight AD patients (5.2% ± 1.2%) with comparable numbers of CD16\(^{+}/\)CD3\(^{-}\) cells present in the analyzed samples and compared K562 lysis of unstimulated, mature NK cells. We demonstrated a significantly lower NK activity in PB of seven early CP (14.3% ± 1.1% lysis of K562, \(P = .02\)), six late CP (14.6% ± 2.4% lysis, \(P = .05\)), and eight AD (7.8% ± 3% lysis, \(P < .01\)) CML patients compared with normals (27.6% ± 4% lysis) (Fig 9).

**DISCUSSION**

We show that an A-LAK population with potent cytotoxicity against NK sensitive and resistant targets, as well as high proliferative capacity, can be generated from the peripheral blood of recently diagnosed chronic phase CML patients.
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The A-LAK cytotoxicity of these early CP CML patients, which is similar to that of A-LAK from normal individuals, diminishes profoundly in patients with either prolonged duration of chronic phase or with advanced disease. The proliferative capacity of A-LAK cells in early CP patients is significantly greater than that of A-LAK from normal individuals and late CP CML patients and diminishes significantly in patients with advanced disease.

The diminution in A-LAK cytotoxicity and proliferation associated with prolonged duration of the disease or progression to advanced disease probably cannot be explained by acquisition of a malignant phenotype, since Southern analysis failed to reveal the presence of the bcr gene rearrangement in CML A-LAK. However, the possibility exists that further testing of the CML A-LAK population with newly developed methods, such as X-linked restriction fragment length polymorphisms, may show evidence of clonality associated with a multistep pathogenesis of malignancy.

In order to exclude the possibility that the differences in cytotoxic and proliferative potential between A-LAK cells generated from the blood of early CP, late CP, and AD CML patients represent an artifact inherent to the culture system that relies on adherence of A-LAK precursors to plastic, we examined the cytotoxic and proliferative capacity of conventionally generated LAK cells in patients with early CP, late CP, and AD CML. LAK cell generation does not require an adhesion step. A similar pattern of diminished cytotoxic and

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**Fig 4.** Southern analysis of CML myeloid precursor cells' and CML A-LAK cells' DNA in three representative early CP (lanes A through C), late CP (lanes D through F), and AD (lanes G through I) CML patients. Genomic DNA was digested with Bgl II and probed with a 1.95 Bgl II/HindIII probe. The arrows at the left side show the bcr gene rearrangement present in myeloid cells of each patient; G, the germ line DNA band position.

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**Fig 5.** Cytotoxicity of day-14 A-LAK cells generated in seven early CP patients (○), eight late CP patients (●), 10 AD CML patients (▲), and 17 normal individuals (□) against an NK-sensitive target K562 and an NK-resistant target Raji. Cytotoxicity data are presented as mean ± SEM percent lysis at four different effector: target ratios. Differences between early CP CML patients and normals are not significant. Differences between normals and late chronic phase patients are significant at the P = .01 (*), .05 (**) levels. Differences between normals and AD CML patients are significant at the P < .01 (#) level.

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**Fig 6.** Comparison of cytotoxic activity of A-LAK cells from three untreated early CP CML patients (○) and four hydroxyurea-treated early CP CML patients (●) against the NK-sensitive target K562 and the NK-resistant target Raji. Cytotoxicity data are presented as mean ± SEM percent lysis at four different effector: target ratios. Differences in cytotoxicity between treated and untreated patients are not significant.
AD CML patients treated with hydroxyurea only target K562 and the NK resistant target Raji. Cytotoxicity data are extensively treated AD CML patients against the NK-sensitive K562 cells. Sem percent lysis at four different effector:target ratios. Differences in cytotoxicity between patient groups are not significant.

Profoundly impaired cytolytic activity in patients with advanced disease. Patients with either late CP CML or advanced disease. Differences in adherence of A-LAK precursors to plastic in various stages of disease are probably not caused solely by differences in adherence of A-LAK precursors to plastic in patients with either late CP CML or advanced disease.

Suppressive effects on immune function and NK activity induced by in vivo or ex vivo administration of cytoreductive agents have been reported. To our knowledge, such suppression has not been documented for hydroxyurea. In this study, we were unable to demonstrate differences in cytotoxicity and proliferation between A-LAK cells generated in untreated and treated early CP patients, nor did we find significant differences between AD patients treated with hydroxyurea alone or in combination with other agents. Three of four patients with CML in blast crisis were studied 1, 3, and 6 months after diagnosis and received hydroxyurea therapy only. No differences could be found in cytotoxic or proliferative capacity of A-LAK cells generated in these relatively untreated patients and that of A-LAK cells generated in the seven additional AD CML patients who received either prolonged or more extensive therapy. The A-LAK proliferation and cytotoxicity in these relatively untreated patients with CML in blast crisis was also significantly diminished in comparison with seven early CP patients, suggesting that advanced disease stage, rather than duration or extent of therapy, influences cytotoxicity.

Several studies show that in vivo and in vitro stimulation of PB lymphocytes with rIL-2 results in the generation of a heterogeneous population of effector cells capable of mediating LAK effector activity. This heterogeneity is partly related to differences in the quantitative expression of CD56 surface antigen. Although LAK activity can be mediated by different types of lymphocytes, it has been demonstrated that the most potent LAK effector activity is mediated by CD56<sup>bright</sup> cells. CD56<sup>bright</sup> cells also showed increased proliferation upon culture with rIL-2 compared with other lymphocyte populations. We demonstrate in this report that a highly significant correlation exists between the quantitative expression of the CD56 surface antigen on A-LAK effector cells generated in patients with CML at different stages of disease and A-LAK cytotoxic and proliferative response upon stimulation with rIL-2.

Although cells of early CP CML patients grown in A-LAK culture for 14 days achieve high levels of cytotoxicity, spontaneous cytotoxicity of resting, mature NK cells circulating in the peripheral blood of early CP CML patients is low, and no change in cytotoxicity of peripheral blood NK cells can be demonstrated with progression of disease. The reason for the disparity between lytic activity of early CP CML A-LAK and mature NK cells obtained from peripheral blood is unclear. The precursor of the human A-LAK cell population has not been conclusively identified but may be different from the mature peripheral blood NK cell. Indeed, studies in mice show that the generation of A-LAK cells is not influenced by deletion of mature NK cells with the monoclonal antibody anti-asialo GM-1 nor by activation of mature NK cells with poly I:C. A-LAK generation is also as efficient in mice with low spontaneous NK activity as in mice with normal or increased NK activity, suggesting that murine A-LAK may arise from a precursor different from the mature peripheral blood NK cell and that NK activity in the peripheral blood does not necessarily predict the cytotoxic potential of cells obtained after A-LAK culture. Recent studies in humans demonstrate that the CD56<sup>+</sup>/CD16<sup>-</sup> and CD56<sup>+</sup>/CD16<sup>dim</sup> subsets of PB NK cells lack spontaneous NK cytotoxicity against K562 targets but respond preferentially to rIL-2 stimulation with increased cytotoxicity and increased proliferation. In contrast, the mature CD56<sup>+</sup>/CD16<sup>bright</sup> NK cell subset (greater than 90% of PB NK cells), which is responsible for most of the spontaneous NK activity in unstimulated PB lymphocytes, demonstrates...
A-LAK function diminishes with advancing CML

significant diminished response to stimulation with rIL-2. These data support the hypothesis that the highly cytotoxic and proliferative LAK and A-LAK cells may be derived from a small subset of immature NK cells that are CD16dim or CD16-, rather than from the CD16+ mature PB NK cells. In order to characterize further the killer cell defect associated with disease progression in CML, it will be necessary to identify and purify different lymphocyte subpopulations from the peripheral blood of patients with CML in different phases of the disease and to examine their response to in vitro rIL-2 stimulation. Such studies will permit us to attribute the diminished cytotoxic and proliferative capacity of day-14 A-LAK cells associated with disease progression in CML to either an inherent decreased response of the progenitor cells to stimulation with rIL-2 or to a decreased presence of such progenitor cells in the peripheral blood of patients with advanced disease or prolonged disease duration.

In this study, we demonstrate normal A-LAK cytotoxicity in early CP CML patients, but a diminution in both A-LAK cytotoxicity and proliferation with prolonged duration of chronic phase or occurrence of advanced phase. These findings suggest that defects in cytotoxic potential of killer cells are associated with the occurrence of accelerated phase and blast crisis in patients with CML. The disparity between the high cytotoxic capacity of early CP CML A-LAK cells and mature unstimulated peripheral blood NK cells shows that the A-LAK culture method reveals a population of rIL-2-stimulated killer cells with cytotoxic potential not reflected in conventional studies of unstimulated, mature peripheral blood NK cells. Further study of the A-LAK population and its precursors in patients with leukemia may lead to a better understanding of mechanisms underlying tumor progression.

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REFERENCES


