Lymphokine-activated killer (LAK) cells and cytokines synergize to kill clonal cells in acute myeloid leukemia (AML) in vitro

SUSANNE BRAUN¹, HELMUT H. GERHARTZ² and HELGA M. SCHMETZER³,*

¹Charité, Campus Virchow-Klinikum, Experimental Surgery, Humboldt University of Berlin, Germany
²Municipal Hospital Meissen, Dept. for Internal Medicine, Germany
³Klinikum Großhadern, Med. Dept. III, University of Munich, Germany

Abstract—We studied the influence of autologous lymphokine-activated-killer (LAK) cells on the survival of clonal and CD34-positive bone marrow (BM) cells from patients with acute myeloid leukemia (AML) in a coculture assay in vitro. (1) LAK cells were grown in the presence of IL-2, in some cases additionally with IL-6. (2) These cytotoxic cells were cocultured with (untreated or cytokine pretreated) AML-BM cells obtained at different stages of the disease. Therefore BM cells were (a) either frozen in liquid nitrogen or (b) precultured for 14 days with cytokines: IL-1β, IL-3, IL-6, erythropoietin (EPO), stem cell factor (SCF) with (‘Cytok1’) or without granulocyte macrophage colony stimulating factor (GM-CSF) (‘Cytok2’) or with no added cytokines (‘ISC/FCS’) as a control. (3) Southern blot analysis was used to detect clonal BM cells. At diagnosis, 76 of 151 cases (50%) studied showed clonal gene rearrangements in marker genes. (4) Southern blot analysis and flow cytometry were used to compare the amount of clonal and CD34 positive BM cells before and after coculture procedures. Coculture experiments with untreated BM and autologous LAK cells led to a reduction of clonal cells in 2 of 5 cases at diagnosis, in 11 of 17 BM samples in complete remission but not in the one case studied at relapse. Similar results were found if precultured AML cells (with or without cytokines) were cocultivated with LAK cells. However the cytotoxic effect of LAK cells was more pronounced if cytokines (especially GM-CSF and SCF) were comprised.

Our data indicate, that (1) clonality in AML can be demonstrated by Southern blot analysis; (2) CD34 positive cells in AML are clonal, gene rearranged cells; (3) clonal cell populations persist in BM during complete remission and relapse in most of the patients; (4) incubation of AML-BM cells with LAK cells lead to a reduction of clonal, rearranged cells in 11 of 17 AML cases in complete remission, but only in 2 of 6 cases at diagnosis or relapse; (5) AML cells can be sensitized to the
LAK cell treatment by preincubation of AML-BM cells with cytokines (IL-1β, IL-3, IL-6, SCF, EPO and GM-CSF) or by adding SCF to the coculture conditions.

Southern blot analysis and flow cytometry are appropriate methods to detect and quantify leukemic disease. Cytokines and LAK cells synergize to kill AML blasts \textit{in vitro}. This is a feasible approach to immunotherapy of AML and merits further investigations.

\textit{Key words}: AML; LAK cells; cytokines; residual disease; clonality; flow cytometry; \textit{in vitro} treatment.

\section*{INTRODUCTION}

Acute myeloid leukemia (AML) results in accumulation of leukemic blasts through clonal proliferation of one abnormal progenitor cell. These blasts usually are morphologically and biologically homogeneous \cite{1}. Leukemic cell populations can be identified by flow cytometry using a panel of different antibodies \cite{2}. Most of the AML cases show a CD34 positive blast phenotype. Clonal cell populations can be identified by cytogenetics, polymerase chain reaction or by Southern blot analysis \cite{3, 4}. In about one third of all AML cases, gene rearrangements in the T-cell receptor (TcR) or the Immunoglobulin (Ig) genes are found in clonal cells \cite{5, 6}. Further gene rearrangements in AML are observed in the GM-CSF-, G-CSF- or IL-3-genes \cite{7–9}. Gene rearrangements in the retinoic acid receptor alpha (RARα) gene are typical for promyelocytic leukemia (AML-M3). Therefore gene rearrangements can be used to study the presence and the amount of clonal, gene rearranged cells in the course of the disease \cite{10}.

About 70\% of patients with AML in complete remission normally relapse during the next two years. Therefore, residual leukemic cells must have survived \cite{11}. In patients who do not relapse, mechanisms must exist which inhibit or even eradicate those leukemic cells. Beside cellular mechanisms several soluble factors and cytokines like IL-2 or Interferons seem to play a role in the suppression of leukemic cells \cite{12–14}. It has been routine practice that animals have been treated in some trials by several cytokines in order to find tumortoxic factors or to test the efficiency of factors in restoring normal BM cells \cite{15, 16}. Moreover, IL-3, GM-CSF or G-CSF are reported to be regulators of the proliferation of leukemic cells \cite{17}. Preclinical data showed a positive synergistic effect of SCF, IL-1β, IL-3, IL-6 and EPO on the proliferation of healthy hematopoietic progenitor cells \cite{14}. Several cytotoxic mechanisms mediated by natural killer cells are known that can suppress leukemic growth \textit{in vivo}. Analog findings were observed with LAK cells, activated T/natural killer (NK) cells \textit{in vitro} \cite{18–20}. However, the proof for an activity of killer cells against leukemic cells at diagnosis or in the course of the disease is still lacking. Based on these findings we investigated the influence of LAK cells on clonal leukemic BM cells. Initially, the LAK cells were cocultured with untreated BM cells of AML patients at diagnosis or in the course of the disease. Subsequently, we investigated the influence of combinations of growth factors including GM-CSF and SCF in ‘priming’ AML-BM cells in order to amplify the effect of LAK cells in