Peripheral Blood and Bone Marrow Immunophenotypic and Functional Modifications Induced in Acute Leukemia Patients Treated with Interleukin 2: Evidence of in Vivo Lymphokine Activated Killer Cell Generation

Robert Foa, Anna Guarini, Anna Gillio Tos, Silvia Cardona, Maria Teresa Fierro, Giovanna Meloni, Silvia Tosti, Franco Mandelli, and Felice Gavosto

Dipartimento di Scienze Biomediche e Oncologia Umana, Sezione di Clinica Medica, University of Turin, and Dipartimento di Biopatologia Umana, Sezione di Ematologia, University of Rome, Italy

ABSTRACT

The effect of treatment with interleukin 2 (IL2) on the phenotypic and functional immune system of acute leukemia patients was investigated. Fifteen acute myeloid leukemia and acute lymphoid leukemia patients with evidence of persistent disease were further subdivided into two groups according to the percentage of bone marrow (BM) blasts: group a had 6–15% blasts and group b had 30–65%. Following two cycles of IL2 (Glaxo Imb, Geneva, Switzerland) given i.v. by continuous infusion at escalating doses, no major changes in the proportion of CD3+, CD4+, and CD8-positive cells were encountered in the blood or in the marrow of either group of patients. When these could be restaged after four cycles of IL2, a significant increase of CD3+ and CD4+ cells was documented in the peripheral blood (PB), as well as a significant increase of CD3+ cells in the BM. Irrespective of the number of cycles administered, the proportion of CD16+ cells increased significantly in the blood in both groups of patients and in the marrow of group a patients only. The expression of CD25 was significantly enhanced in all samples tested.

Following IL2 administration, an enhancement of the natural killer compartment was documented. This was consistently more evident in patients with more limited disease. A significant amplification of the in vitro-induced lymphokine-activated killer function was noted in the BM of the treated patients. Furthermore, we documented the presence both in the PB and in the BM of “spontaneous” lymphokine-activated killer cells generated in vivo following IL2 administration.

These results demonstrate that in acute leukemia of both myeloid and lymphoid origin, treatment with IL2 is capable of inducing profound immunophenotypic and functional modifications in PB and in BM lymphocytes, particularly in patients with more limited disease. The evidence of the in vivo activation of cytotoxic cells, particularly in the BM, may help to explain the clinical responses preliminarily observed in individual acute leukemia patients.

INTRODUCTION

Following a 2–7 day preincubation with IL2, mouse and human lymphocytes are capable of lysing both allogeneic and autologous tumoral cells (1, 2). This cytotoxic activity is mediated by the so-called LAK cells. These findings and the evidence that LAK effectors may display an antitumor activity in animal models (3, 4) have represented the prerequisite base-

Received 8/9/90; accepted 11/6/90.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by Istituto Superiore di Sanita, Rome, and by Ministero della Pubblica Istruzione, Rome. A. G. T. is in receipt of a fellowship from Associazione Italiana per la Ricerca sul Cancro, Milan.

2 To whom requests for reprints should be addressed, at Dipartimento di Scienze Biomediche e Oncologia Umana, Sezione di Clinica Medica, University of Turin, Via Genova 3, 10126 Torino, Italy.

3 The abbreviations used are: IL2, interleukin 2; LAK, lymphokine-activated killer; NK, natural killer; cpm, counts per min; PB, peripheral blood; BM, bone marrow.

964
“spontaneous” and IL2-induced LAK activity on both PB and BM cells.

Cell Separation. Mononuclear PB and BM cells were obtained after fractionation on a Lymphoprep (Nycomed AS, Oslo, Norway) gradient. The cells recovered from the interface were washed twice and resuspended in RPMI 1640 (Flow Laboratories, Opera, Italy) supplemented with 10% heat-inactivated fetal bovine serum (Flow).

Immunophenotyping. On PB and BM mononuclear cells, the expression of the following antigens was monitored: Leu4 (CD3), Leu3 (CD4), Leu2 (CD8), Leu1 (CD16), and IL2-receptor (CD25; Becton Dickinson, Mountain View, CA). All reagents used were directly labeled and the percentage of positive cells was analyzed using a FACSscan flow cytometer (Becton Dickinson) or under a fluorescence microscope.

Generation of LAK Cells. LAK cells were generated by incubating mononuclear cells at the concentration of 1.5 x 10⁶/ml in RPMI 1640 (Flow) supplemented with 10% heat-inactivated fetal bovine serum (Flow), in the presence of 100 units/ml of recombinant IL2 (GlaxoImb) for 7 days at 37°C with 5% CO₂ in humidified air. In a previous study (10), we reported that under our experimental conditions the presence of 1000 units/ml of IL2 did not modify significantly the generation of LAK cells. The preculture time was prolonged to 7 days to allow an optimal generation of LAK effectors also from samples containing variable proportions of blasts (11, 18). LAK cell activity was based on the percentage of ⁵¹Cr release (see below) by the NK-resistant B-lymphoid cell line Raji.

“Spontaneous” LAK Cells. To assess whether the administration of IL2 was capable of inducing in vivo the generation of LAK cells, the “spontaneous” lytic activity against Raji cells was also tested on PB and BM lymphocytes before and after treatment in the absence of any further in vitro preincubation with IL2.

NK Activity. The NK activity of PB and BM mononuclear cells was measured using the classic ⁵¹Cr release assay against the K562 cell line (see below).

Cytotoxic Assay. A standard ⁵¹Cr release assay was used. Target cells (5 x 10⁴) were incubated with 100 μCi of ⁵¹Cr (Na ⁵¹CrO₄; Dupont, New England Nuclear Products Division, Florence, Italy) for 1 h at 37°C and then washed twice with complete medium. A total volume of 150 μl of complete medium containing 2 x 10⁵ target cells and various numbers of effector cells at final effector:target ratios of 100:1, 50:1, 25:1, and 12:1 were placed into round-bottomed microtiter plates (Flow). The plates were incubated at 37°C for 4 h and then centrifugated at 1300 x g for 10 min. An aliquot (100 μl) of the supernatant was collected and counted in a gamma scintillation counter. All experiments were performed in triplicate, and the percentage of ⁵¹Cr release was calculated according to the following formula:

\[(E - S)/(M - S) \times 100\]

where \(E\) is the mean cpm release in the presence of effector cells, \(S\) is the mean cpm spontaneously released by the target cells incubated with medium alone, and \(M\) is the mean cpm release of 100 μl of resuspended target cells.

The results reported always referred to a final effector:target ratio of 100:1. Only results of experiments in which the value of \(S/M\) release was less than 25% are included.

RESULTS

Immunophenotypic Characterization. The percentage of PB CD3-, CD4-, and CD8-positive cells in patients with more limited disease (6-15% BM blasts) did not change significantly after 2 cycles of IL2 therapy: 58 ± 11.2% (post-IL2) versus 58.7 ± 12% (pre-IL2); 36.3 ± 16% versus 31.8 ± 6.5% and 24 ± 8.6% versus 25.5 ± 8.6%, respectively (Fig. 1a). On the contrary, the expression of the CD16 and of the CD25 antigens increased and were significantly higher (P < 0.001) compared with the basal values: 25.3 ± 9.6% versus 7.7 ± 4.1% and 13.1 ± 6.8% versus 0, respectively (Fig. 1a). BM cells showed, following IL2 administration, a small increase of CD3+ cells (15.1 ± 5.2% versus 11.3 ± 7.9%), but a significant increase of the CD16 antigen (14.7 ± 9.3% versus 6.0 ± 4%, P < 0.05) and of CD25+ cells (2.8 ± 2.3% versus 0, P < 0.01) (Fig. 2a).

Also in the PB lymphocytes of patients with 30-65% blasts in the BM we observed few changes in the expression of CD3, CD4, and CD8 after 2 cycles of IL2 therapy: 71.7 ± 9.4% versus 66.2 ± 11%; 48.5 ± 12.6% versus 43.7 ± 14.2%, and 21.8 ± 3.7% versus 23 ± 3%, respectively (Fig. 1b). CD16- and CD25-positive cells increased significantly (P < 0.02 and P < 0.005) their expression after therapy: 26.7 ± 11.9% versus 11.5 ± 7.5% and 22.1 ± 15.3% versus 2 ± 1.8%, respectively (Fig. 1b). In the BM, little or no variations were observed in the expression of CD3 (12.8 ± 7.5% versus 12.5 ± 6%) and CD16-positive cells (9.2 ± 5.2% versus 5 ± 5%), whereas the CD25 antigen after IL2 administration was significantly (P < 0.02) higher compared with the basal values: 5 ± 4.5% versus 0 (Fig. 2b).

NK Activity. An enhancement of the NK cell activity was consistently observed after IL2 treatment (Fig. 3). In both PB
and BM lymphocytes of patients with 6–15% blasts, the NK activity increased considerably compared with the pre-IL2 values: 48.5 ± 25.2% versus 16.3 ± 9.4% (P < 0.005) and 18.9 ± 16.6% versus 9.7 ± 9%, respectively (Fig. 3a). In patients with a larger tumoral mass, the increment of NK activity was less evident in both PB and BM cells: 23.4 ± 12.2% versus 17.3 ± 24.8% and 7.1 ± 6.5% versus 4.4 ± 3.6%, respectively (Fig. 3b).

**In Vitro-Induced LAK Activity.** Prior to starting treatment, a satisfactory capacity of generating in vitro LAK activity was present in PB cells of all patients. In patients with 6–15% blasts, the mean basal value was 37.1 ± 2.1%, whereas after IL2 therapy the LAK function increased to 46.6 ± 10.5% (Fig. 4a). Also in the other group of patients we observed a high basal LAK capacity (47.6 ± 29.6%), which did not increase further following IL2 therapy (44.1 ± 12.7% (Fig. 4b). After IL2 administration, a significant increase in LAK function was observed in BM lymphocytes, particularly in patients with more limited disease, in whom this rose from 12 ± 13.8% to 42.3 ± 20.8% (P < 0.005) (Fig. 4a). This enhancement of the LAK compartment was also documented in patients with more evident disease (31.2 ± 23.9% versus 10.4 ± 9.2%, respectively, P < 0.05) (Fig. 4b).

**“Spontaneous” LAK Activity.** Following in vivo administration of IL2, acute leukemia patients were capable of generating a “spontaneous” LAK activity. This was always absent before IL2 administration. After IL2 therapy, a “spontaneous” in vivo LAK function was found in the PB of all but one patient with 6–15% blasts (mean 11.7 ± 8.6%, P < 0.002) and of all patients with 30–65% blasts (6.1 ± 3.6%, P < 0.0001) (Fig. 5, a and b). Also in BM cells we could document the presence of “sponta-

**DISCUSSION**

Treatment of cancer patients with IL2-mediated immunotherapy has allowed encouraging results, particularly in some categories of solid tumors (5–7). Though the exact mechanism(s) by which this new approach has proved effective still needs to be fully clarified, the evidence of in vivo-induced modifications of the immune system (19–24), namely of the cytotoxic compartment, as well as the release of different cytokines (25, 26), suggests that these biological changes may play a role in tumor regression. The recent evidence that the administration of IL2 may also be successfully used in the management of a proportion of acute leukemia cases (13, 15–17) has opened the question of whether the immune machinery in this category of patients, often considered impaired, is sufficiently preserved to allow a phenotypic and functional activation following IL2 treatment, thus offering a clue to the mechanism(s) of blast clearing.

The results of our study demonstrate that in acute leukemia patients with evidence of disease, the administration of IL2 is capable of an overall boosting of the NK and LAK systems. This activation occurs in both myeloid and lymphoid leukemias, suggesting, therefore, that in these patients a relatively effective cytotoxic compartment is still present. In agreement with preclinical data (11, 12), the activation of the cytotoxic machinery could be documented also in patients who, at the time of the study, displayed a high percentage of leukemic cells in the marrow. Furthermore, this often occurred in the BM, as well as in circulating lymphocytes.

When the distribution of the T-lymphocyte subsets was analyzed, the expression of CD3, CD4, and CD8 did not vary significantly after two cycles of IL2. Only the CD25 (IL2 receptor) antigen increased significantly. These data are in agreement with those reported by other authors in solid tumor patients treated with IL2 (23). However, in patients in whom the clinical situation allowed to continue treatment with IL2, a significant increase in the percentage of circulating CD3+ and CD4+ lymphocytes was documented after 4 cycles, paralleled by an increase and normalization of the CD4:CD8 ratio. The prolonged treatment with IL2 also allowed a significant increase in the percentage of CD3 cells infiltrating the BM. These findings suggest that in acute leukemia patients, an amplification of the T-cell compartment requires a longer time period.
and/or a prolonged exposure to IL2. In accordance with the enhancement of the NK activity, treatment with IL2 resulted in a rapid and marked increase of CD16+ cells both in the blood and in the marrow, which occurred already after 2 cycles of treatment.

When the cytotoxic function was analyzed in detail and in relation to the clinical status of the patients at the time of treatment, several peculiarities emerged. First, IL2 treatment showed a generalized capacity of boosting the NK function of acute leukemia patients; this effect was, however, significantly greater in patients with more limited disease. In view of the suggestion that monitoring of the circulating NK activity in acute leukemia patients may bear a prognostic significance (27) and that treatment with IL2 alone may be effective mainly or only in cases with a small proportion of residual blasts (13, 15–17), the evidence of a differential activation of the NK compartment by IL2 may be of clinical relevance.

As mentioned above, marked modifications of the LAK compartment were documented. In view of the disease under study, it is of interest that these were predominantly observed in the BM. A significant enhancement of the LAK activity was documented in BM lymphocytes of patients with advanced disease and, more so, of patients with limited disease. More relevantly, the administration of IL2 was capable of inducing the generation in vivo of “spontaneous” LAK effectors that could be detected in the PB and in the BM of both groups of patients. These data demonstrate that LAK cells can be induced in acute leukemia patients and suggest that this event may take place in the BM, which is the site of origin of the disease, as well as the main sanctuary of resistance and of primary recurrence of disease. To our knowledge, this is the first demonstration that LAK cells may be found in the BM following in vivo administration of IL2, thus stressing the potentiality of treating by immunotherapeutic means acute leukemia blasts in their most “physiologic” site. The recent demonstration that residual leukemic blasts may be eliminated following the in vivo administration of IL2 alone (13, 15–17) confirms, on clinical grounds, the relevance of the immunological modifications described in this study.

The final goal of recognizing biological parameters that may allow identification of individual patients potentially responsive to this form of treatment was unsuccessful, as reported for renal cell carcinoma patients (23). In view of the recent evidence that in acute leukemia cases at diagnosis and in relapse the in vitro IL2-induced LAK function may not always kill autologous blasts,4 work is in progress to ascertain if and to what extent in these patients the cytotoxic activity documented in vivo following IL2 administration is specifically directed against the autologous neoplastic population and not only against an unrelated target.

REFERENCES


Peripheral Blood and Bone Marrow Immunophenotypic and Functional Modifications Induced in Acute Leukemia Patients Treated with Interleukin 2: Evidence of in Vivo Lymphokine Activated Killer Cell Generation

Robert Foa, Anna Guarini, Anna Gillio Tos, et al.


Updated version Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/51/3/964

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.