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# The Role of Interleukin-1 Inhibitors on Acute Myeloblastic Leukemia Blast Proliferation; Future Potential for Biotherapy

Cavit ÇEHRELİ, G. Hayri ÖZSAN, Fatih DEMİRKAN, Halil ATEP, Bülent ÜNDAR, Faize AKYOL, İlhan ÖZTOP, Uğur YILMAZ

Division of Hematology-Oncology, School of Medicine, Dokuz Eylül University, İzmir, TURKEY

## SUMMARY

The effect of interleukin-1 (IL-1) as an autocrine growth factor on the proliferation of the acute myeloblastic leukemia (AML) blasts was studied. Bone marrow specimens were obtained from nine patients with different subgroups of AML. IL-1 receptor antagonist (IL-1RA) and IL-1  $\beta$  neutralizing antibody (IL-1 $\beta$  NA) alone or in combination were added to the culture mediums of the AML blast cultures for the detection of their inhibitory effect on AML blast cell proliferation and colony formation. Average colony numbers in the IL-RA, IL- $\beta$ NA, and IL-IRA plus IL- $\beta$ NA included culture flasks, were  $63.7 \pm 21.5\%$ ,  $69.5 \pm 19\%$ ,  $53.4 \pm 23.7\%$ , respectively, as compared to those of the control ( $p < 0.01$ ). Inhibition of colony formation by IL-IRA plus IL- $\beta$ NA was more prominent than by IL- $\beta$ NA alone ( $p < 0.01$ ). No correlation between the inhibition of AML blast colony formation and FAB AML subgroups was seen.

Result: Both IL-1RA or IL- $\beta$ NA or in combination induced varying degrees of inhibition on blast colony formation. IL-1 inhibitory molecules could be considered as an alternative therapy for AML in patients whose blast cells are sensitive to IL-1 inhibition.

Key Words: Acute myeloblastic leukemia, IL-1, IL-1 receptor antagonist, IL-1 $\beta$  neutralizing antibody, Blast culture.

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## INTRODUCTION

Acute myeloid leukemia (AML) is a clonal hematologic disorder characterized by the abnormal proliferation of myeloid progenitors and lack of maturation. Although AML blasts, as in other myeloid precursor cells, require hemopoietic growth factors elaborated by the bone marrow stromal cells and accessory cells, some of the blast cells have the capacity for autonomous growth and require no additional growth factors<sup>[2]</sup>. The secretion of some hemopoietic growth factors, such as granulocyte/macrophage colony stimulating factor (GM-CSF) and interleukin-1(IL-1), by the AML blasts has been demonstrated in several studies<sup>[3,4]</sup>. In vitro studies have shown that the IL-1 could stimulate growth of AML blast cells by both paracrine and autocrine pathways<sup>[5,6,7]</sup>; and addition of IL-1 receptor antagonist (IL-1 RA), as well as soluble interleukin-1 receptor, both of which antagonizes IL-1, into the culture media inhibits proliferation of leukemic blast cells<sup>[8]</sup>.

IL-1 is one of the prototypic multifunctional cytokines, and unlike the other colony stimulating factors, can affect almost all types of cells and induce its effect frequently with other cytokines and mediator molecules. The IL-1 family contains IL-1a and IL-1 $\beta$  as agonists and IL-IRA as an antagonist<sup>[9]</sup>. IL-1a and IL-1 $\beta$  are elaborated as precursor molecules. While IL-1a as a precursor has biological activity, IL-1 $\beta$  as a precursor acquires its

biological activity only after conversion into its active form by the IL-1 $\beta$  converting enzyme(ICE)<sup>[10,11]</sup>.

The aim of this article is to report the results of the effects of the IL-1 $\beta$  neutralizing antibodies (IL-1 $\beta$ NA), IL-IRA and a combination of IL-1 $\beta$ NA and IL-IRA on AML blast cell proliferation in culture mediums containing GM-CSF, and to investigate the proliferative activities of blast cells against these inhibitory cytokines for their potential use in the biological treatment of patients with AML.

## MATERIALS and METHODS

### Collection of Blast Cells

Nine patients with newly diagnosed different FAB subgroups of AML were seen in the DEU Hospital as a part of the study (Table 1). 3 to 5 ml bone marrow specimens, containing 500 U preservative-free heparin, were obtained from the patients with different FAB subgroups of AML and diluted with equal V/V Isocove's Modified Dulbeccos Medium (IMDM) (Sigma Chemical Company St. Louis, MO, USA) and layered on Histopaque 1077 (Sigma Chemical Company St. Louis, MO, USA) and centrifuged on 1600 rpm for 30 minutes. Mononuclear cells (MNCs) obtained from the interphase were resuspended in 5 ml of a-medium (Biocrom K.G. Seromed, Berlin, Germany) containing 10% fetal calf serum (FCS) (Biocrom K.G. Seromed, Berlin, Germany). The MNCs were

Table 1. Patients characteristics

Patient	Age/Sex	FAB category	Hb (g/dl)	WBC (x10 <sup>9</sup> /L)	Platelets (x10 <sup>9</sup> /L)	% Blasts in BM
1	55/F	M1	10.0	140	30	95
2	35/M	M2	8.4	20.5	66	95
3	83/M	M2	10.8	43.1	94	43
4	60/M	M2	7.6	7.1	127	40
5	53/M	M3	9.1	1.0	25	92
6	35/F	M3	9.0	2.0	42	80
7	46/M	M5	7.3	17	35	93
8	36/M	M5	10.8	7.6	44	60
9	40/F	M5	7.6	13	20	92

FAB: French-American-British, Hb: Hemoglobin, WBC: White blood cells, BM: Bone marrow, F: Female, M: Male

washed twice by centrifugation on 1200 rpm for 10 minutes and resuspended with a-medium.

The MNCs were depleted from adherent cells by incubation in a a-medium containing 10% FCS in 25 cm<sup>2</sup> plastic culture flasks (Corning N.Y., USA) in a humidified atmosphere of 5% CO<sub>2</sub> with air, at 37°C for 1 hour. Following incubation, nonadherent MNCs were collected and resuspended in 10% FCS in a a-medium. The percentages of CD14+ cells in mononuclear cells before and after incubation were detected by flowcytometry. A decrease in the CD14+ cells to less than 3% was accepted as a satisfactory depletion.

The nonadherent cell population was also depleted from the T lymphocytes by E rosetting with sheep red blood cells. A decrease in the T lymphocytes to less than 3% was accepted as satisfactory.

#### **AML Blast Culture**

1x10<sup>5</sup> nonadherent and T-cell depleted MNCs/mL were plated in plastic dishes (Falcon, Becton Dickenson, Plymouth, England) containing 2 mL of 0.8% methylcellulose (Sigma Chemical Company St. Louis, MO, USA) in a-medium supplemented with 20% FCS, 25 mg/mL L-Glutamin (Sigma Chemical Company St. Louis, MO, USA) and 15 ng/mL GM-CSF (Shering-Plough, Brinny, Ireland). 200 ng/mL of IL-1βNA, 500 ng/mL IL-1RA (Genzyme, Cambridge, MA, USA) and similar amount of IL-1βNA plus IL-1RA were added in to the three different plastic dishes containing the above mentioned culture medium, respectively. Similar amounts of MNCs were also plated in the same medium having GM-CSF, but neither IL-1βNA nor IL-1RA, as a control culture. All culture dishes were incubated in a humidified atmosphere of 5% CO<sub>2</sub> with air at 37°C for 7 days. Clusters consisting of more than 20 cells were counted as CFU-AML colonies under an inverted microscope.

Following this process the culture contents of each plastic dish were transferred to separate tubes for centrifugation. Cyto centrifuge smears were also prepared from each culture dish and stained with Wright's stain.

Statistical analysis: differences among colony numbers were evaluated by nonparametric (Mann-Whitney) tests.

#### **RESULTS**

Following the adherent cell depletion of the MNC, the percentages of CD14+ cells were found to be 29.1% and 10.5% on patients No 3 and No 6, respectively; but in these patients the values of CD14+ cells before adherent cell depletion were found to be (57% and 21.4% respectively) very high. This was attributed to the aberrant expression of CD14 on leukemic cells in these patients. The viability of the MNCs before plating were found to be more than 90% by the Trypan blue dye exclusion test.

Figure 1 reveals the average percentages of CD2+ and CD14+ cells before and after depletion.

A significant decrease in the colony numbers were recorded in the culture dishes having IL-1βNA (69.5 ± 19), IL-1RA (63.7 ± 21.5) and IL-1βNA + IL-1RA (53.4 ± 23.7) as compared to those of control cultures (p < 0.01) (Figure 2). No significant differences were observed in the colony numbers of the culture dishes containing IL-1RA alone and IL-1βNA plus IL-1RA; but more significant decreases were noticed in the culture dishes in which both IL-1βNA and IL-1RA were included as compared to those of IL-1βNA alone (p < 0.01). A decrease in the colony numbers, varying from 0% to 78%, was observed by the inhibition of IL-1, with above mentioned IL-1 antagonists in the individual patients. Colony inhibition was not found to be homogenous when patients were evaluated according to their FAB subgroups. (Figure 3).

Despite the adherent cell depletion of MNCs, cyto centrifuge smears prepared from AML blast cultures showed 10-15% macrophages in addition to blasts.

#### **DISCUSSION**

The autonomous growth characteristics of some AML blasts are related to the autocrine growth factors released by these cells and show their emergence from more primitive cells during differentiation<sup>[2,3,12,13]</sup>. For this reason autonomous growth characteristics of AML blasts are closely

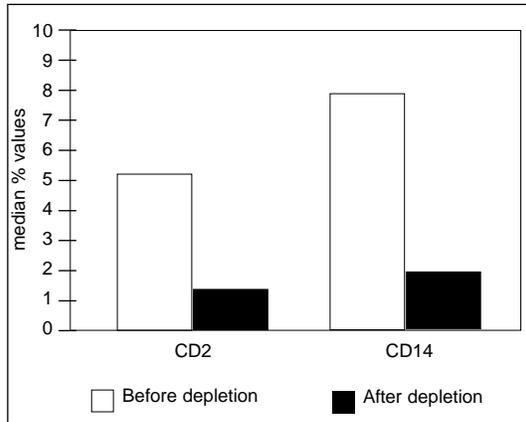


Figure 1. Average percentages of CD2+ and CD14+ cells before and after depletion.

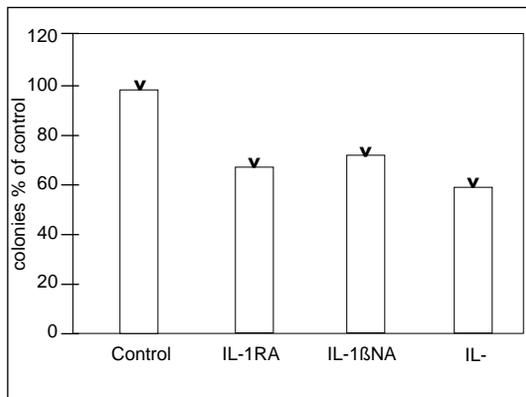


Figure 2. Average decrease in the percentage of colony numbers of culture dishes containing IL-1RA, IL-1βNA and IL-1RA+IL-1βNA as compared to their control cultures. IL-1RA: interleukin-1 receptor antagonist IL-1βNA: interleukin-1 beta neutralizing antibody .

related with a poor prognosis<sup>[14]</sup>. Priesler et al have stated that a short remission duration is related with the expression of IL-1β mRNA in AML blasts<sup>[15]</sup>.

The significant role of IL-1 in AML blast proliferation and the suppressive effect of IL-1 inhibition on AML blast proliferation have been demonstrated by several studies<sup>[4,6,8]</sup>. Estrov et al demonstrated a decrease in colony formation by the addition of IL-1RA and sIL-1β<sup>[8]</sup>. Cozzolina et al demonstrated an inhibition of blast proliferation with IL-1β neutralizing antisera by [<sup>3</sup>H] thymidine uptake test<sup>[4]</sup>. A suppression of AML blast colony formation and the inhibition of blast cell proliferation

by the antagonist molecule of IL-1β NA and IL-1RA were also demonstrated in the present study. In addition, our results showed that the inhibition of colony formation and blast cell proliferation were not related to FAB subgroups, but is possibly due to the differences in the sensitivities of blast cells against the antagonist molecules in each individual patient. Several studies showed that IL-1 increases growth factor synthesis in blast cells<sup>[5,8]</sup>. Some studies suggest that the negative effect of IL-1 inhibition on AML blast proliferation may be due to the inhibition of the GM-CSF release from blast cells<sup>[8,16]</sup>. In our study, despite an addition of exogenous GM-CSF to the culture, the demonstration of inhibition by IL-1 antagonist molecules on blast cell proliferation suggests that the inhibitory effect is not due to a decrease in the GM-CSF release. On the other hand, it was reported that the apoptosis induced by the Fas gene was inhibited by IL-1β<sup>[17]</sup>. Based on these observations and findings, the role of other possible mechanisms should also be considered, in addition to the augmenting effect of IL-1 on the growth factor synthesis in blast cells.

Although a similar amount of inhibitory effects induced by IL-1β NA and IL-1RA on colony formation were observed in the present study, the inhibitory effect of IL-1β NA was increased by the addition of IL-1RA as compared to that induced by IL-1β NA alone. This result may be due to either the inhibition of residual IL-1β by IL-1RA or the inhibition of the stimulatory effect of IL-1 a on AML blast proliferation by IL-1RA. Exogenous IL-1 also increases AML blast proliferation, but the low spontaneous expression of IL-1 a in blasts makes this mechanism unlikely<sup>[4]</sup>. However, despite a depletion of adherent cells before cultures, observation of macrophages in addition to blast cells on cyto-centrifuge smears prepared from AML blast cultures may also be suggestive of a paracrine source of IL-1, and thereby IL-1a.

Our study demonstrated that the inhibitory effect of IL-1βNA, IL-1RA or both were not related to FAB AML subtypes. Differences in the inhibitory effect of these IL-1 antagonist molecules on blast cell proliferation could be possibly due to variations in the sensitivities of blast cells of each indi-

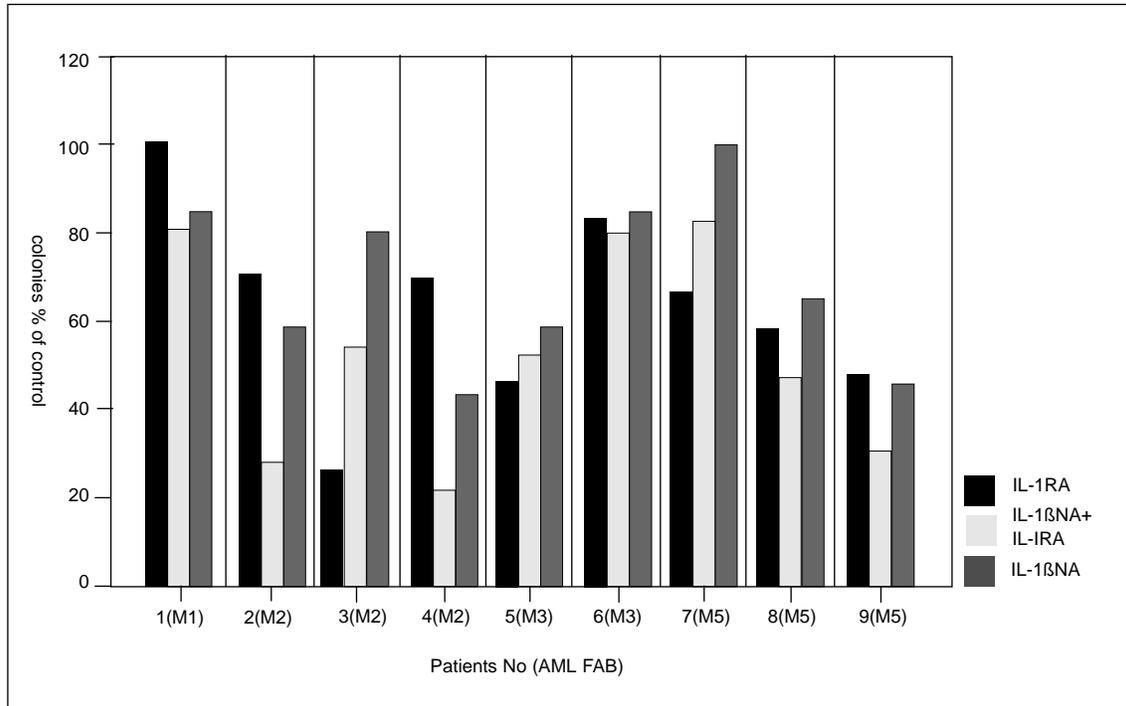


Figure 3. Decreases in the colony numbers of the culture dishes of individual patients with different FAB subgroups of AML.

FAB: French-American-British classification, IL-1RA: interleukin-1 receptor antagonist, IL-1βNA: interleukin-1 beta neutralizing antibody.

individual patient against these molecules. In consideration of IL-1RA and IL-1βNA or both for the biological treatment for AML, these molecules could only be effective in some of the patients with AML whose blast cells are sensitive to IL-1 inhibition.

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**Address for Correspondence:**

Cavit ÇEHRELİ MD

Chief Division of Hematology-Oncology  
Dokuz Eylül University School of Medicine,  
Ynciraltı-İzmir, TURKEY