

Arginase activity alterations in peripheral blood lymphocytes in the human chronic lymphocytic leukemia

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The arginase (EC 3.5.3.1) activity in leukocytes of venous blood is lowered in various diseases affecting white blood cells such as: chronic lymphocytic leukemia, infectious mononucleosis, acute leukemia and the terminal blastic phase of chronic granulocytic leukemia [1, 2]. These observations may reflect quantitative changes mainly in the fraction of granulocytes, which as opposed to mononuclear or blast cells, contribute primarily to the total arginase activity of leukocytes in healthy subjects. Nevertheless, nothing is known about arginase activity in particular types of white blood cells in leukemias.

The physiological function of arginase in normal leukocytes and pathologically altered cells is still unclear. Involvement in the urea cycle should be excluded because of the absence of the other enzymes of the cycle [3]. Recent results show that, in white blood cells, arginase may be involved in regulation of some arginine-dependent biological events *via* modulation of the local arginine availability. Among others, the influence of arginase on DNA synthesis [4] and the immune response [5] was evidenced. Recently, it was also demonstrated that L-arginine is one of the amino acids essential for lymphocyte proliferation [6]. Thus, modulation of lymphocyte growth by arginase should also be considered.

These data together with significant improvements both in the isolation of blood cells [7] and the determination of arginase in small popula-

tions of cells [8, 9], have prompted us to examine arginase activity of lymphocytes in chronic lymphocytic leukemia (CLL)¹.

CLL is a disorder manifested by progressive accumulation of morphologically mature but immunologically incompetent lymphocytes in blood, bone marrow, and lymphatic tissues [10]. As demonstrated by cell surface antigen studies, approx. 95% of the patients have a clonal proliferation of B-lymphocytes (B-cell CLL) with the remainder having T-cell CLL [11]. In both cases the clinical course of the disease progresses from an indolent lymphocytosis, without other evident symptoms and without any need for treatment (stage "0" according to Rai *et al.* [11]), to one of the generalized lymphatic enlargement with concomitant pancytopenia (stage "I - IV" of the disease). Complications of pancytopenia, including hemorrhage and infection, represent a major cause of death in these patients.

The aim of the present study was to follow alterations of arginase activity in leukemic lymphocytes during CLL development and therapeutic treatment.

The activity of arginase was determined in lymphocytes of 69 patients with B-cell CLL and 15 normal donors (control group).

Lymphocytes were isolated from heparinized venous blood by the density gradient procedure according to Bøyum [7], using Gradisol L ("Polfa", Kutno, Poland) as a gradient medium ($d = 1.077 \text{ g/cm}^3$). The separated cells were

¹Abbreviation: CLL, chronic lymphocytic leukemia

washed twice with saline, counted in a Bürker counting chamber or in an electronic counter (Picoscale, "Medicor", Budapest, Hungary), and after centrifugation (10 min, $400 \times g$) resuspended in the homogenization buffer 5 mM Tris/HCl, pH 7.5 and stored at -20°C not longer than for one week. After thawing the cells were homogenized in a Potter Elvehjem homogenizer and the arginase was assayed as previously described [8, 9]. Since arginase from human leukocytes, similarly as the liver arginase, is a metalloenzyme requiring a temperature-dependent binding with Mn^{2+} ions for full activation *in vitro* [3], arginase activity was measured both without Mn^{2+} activation (non-activated enzyme) and after activation with Mn^{2+} at a final concentration of 5 mM, at 55°C for 20 min (Mn^{2+} -activated enzyme). The arginase activity was expressed in the units per 10^{10} cells. One unit (U) is defined as the amount of enzyme that produces 1 μmol of ornithine per min at 37°C .

Leukemic lymphocytes of CLL patients showed a significantly lower total arginase activity in comparison with normal lymphocytes of controls (Fig. 1). In CLL lymphocytes the activity of arginase non-activated by Mn^{2+} was about 3-fold lower than in control cells whereas the activity of Mn^{2+} -activated enzyme was but only about twice as low as in the lymphocytes of healthy subjects. The difference in the reduc-

tion of the activity of non-activated and activated arginase reflects the changes in susceptibility of the enzyme in leukemic lymphocytes to activation by manganese ions. As it is shown in Fig. 2, arginase in leukemic lymphocytes, irrespective of the CLL stage, always was more susceptible to Mn^{2+} activation than the enzyme in normal cells with exception of lymphocytes from patients at early CLL (stage I) in which antileukemic treatment was introduced. The reduction of the total arginase activity in lymphocytes of CLL patients was related to the stage of the disease (Fig. 3). In lymphocytes of the non-treated patients with CLL in stage "0" the mean activity of arginase was on average 3-fold lower than in control cells. In stage I of CLL the mean activity in lymphocytes of non-treated patients was about 4-fold lower than in controls. However, in patients of the same stage of CLL in which standard antileukemic therapy was introduced (the question when to initiate therapy for CLL still remains unresolved) the mean arginase activity was in the range of values found in normal subjects (Fig. 3). These results suggest that antileukemic treatment prevented the decrease of the enzyme activity in patients at stage I of CLL.

In stage II (all patients were receiving antileukemic drugs) the mean arginase activity was still in the range of activities found in non-treated patients in the earlier stages of CLL.

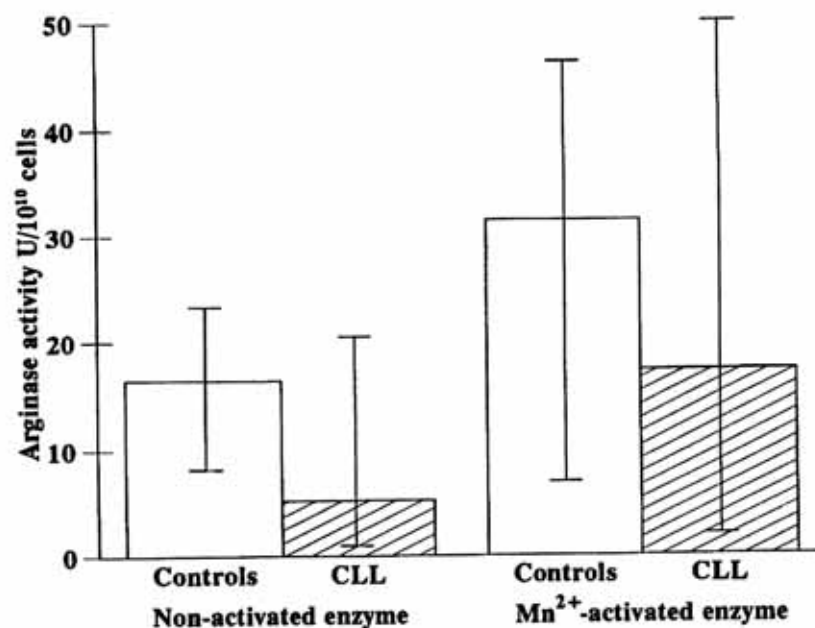


Fig. 1. Total activity of arginase in peripheral blood lymphocytes of CLL patients and normal subjects. Arginase activity was measured before and after activation with MnCl_2 . Mean and range values are given

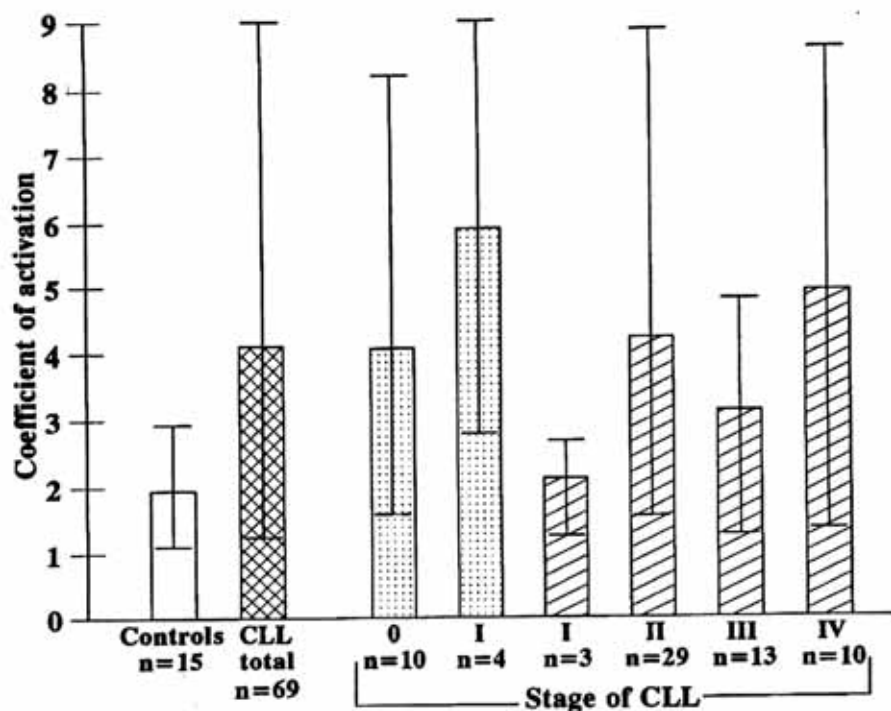


Fig. 2. Activation of arginase with $MnCl_2$ in lymphocytes of CLL patients and normal subjects. Coefficient of activation was calculated as the ratio of the activity of Mn^{2+} -activated arginase to that of non-activated enzyme. \square , Non-treated patients; ▨ , treated patients. Mean and range values are given

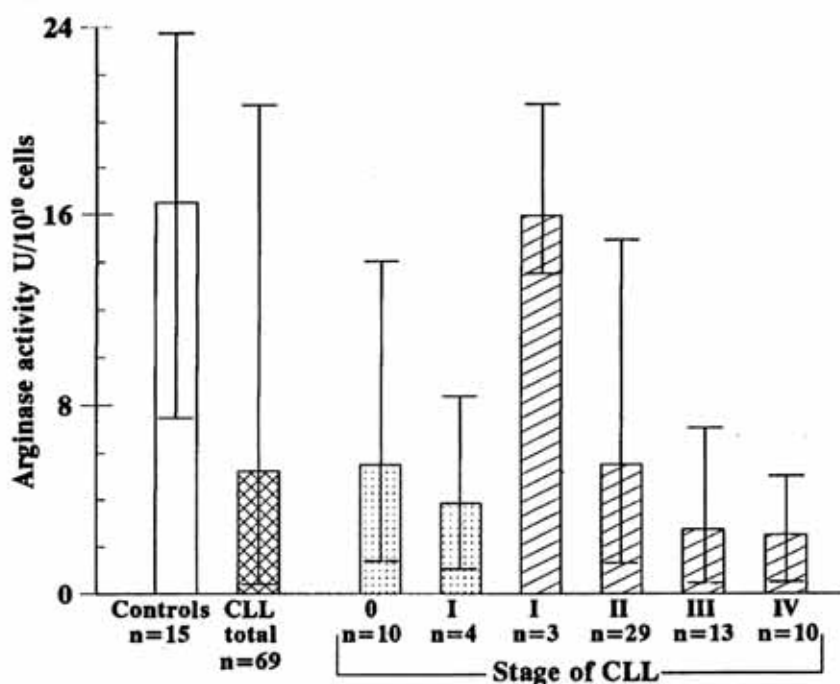


Fig. 3. Total activity of arginase, non-activated by Mn^{2+} , in leukemic lymphocytes in relation to the stage of CLL and its treatment.

\square , Non-treated patients; ▨ , treated patients. Mean and range values are given

In patients at III and IV stages of CLL and maintained on standard chemotherapy, the mean arginase activities in leukocytes were

about 6-fold lower than in the lymphocytes of controls and half those in leukemic lymphocytes of earlier stages of CLL.

Our results demonstrate that CLL development is accompanied by both a progressive deficiency of the total arginase activity in peripheral blood lymphocytes and the decrease of the enzyme susceptibility to Mn^{2+} activation which are delayed, at early but not at late stages of the disease, by antileukemic therapy. Pathophysiological significance of this phenomenon as well as its possible importance in diagnosis of early cases of CLL remains to be established.

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