Patterns of adenosine deaminase, ecto-5'-nucleotidase, poly(A)polymerase and surface light chain expression in chronic lymphocytic leukemias

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Summary. The levels of activity of three enzymes have been measured in the circulating malignant lymphocytes of 47 patients with B chronic lymphocytic leukemia (CLL). These were the purine degradative enzymes, adenosine deaminase (ADA) and ecto-5'-nucleotidase (5'NT) and the enzyme responsible for the polyadenylation of mRNA, poly(A) polymerase. The patterns of activity of the above enzymes and the expression of surface immunoglobulin light chains were examined. A heterogeneity in the specific activity of the enzymes was observed which could not be attributed to variations of the percentage of B lymphocytes. A positive correlation was found between ADA and poly(A)polymerase activity (r = 0.383, p < 0.01). Furthermore, the expression of immunoglobulin light chain phenotype was inversely related to 5'NT specific activity; CLL cases in which less than 20% of the cells expressed L chain phenotype, presented 5'NT specific activity of 16.7 ± 3.3 (S.E.) nmol/h/10⁶ cells, whereas in CLL cases with more than 20% of the cells expressing this phenotype the enzyme specific activity was 4.8 ± 1.6 (S.E.) nmol/h/10⁶ cells (p < 0.02). These findings suggest that the simultaneous determination of enzymatic activities and immunological markers, might be useful in defining subsets in CLL and the subsequent clinical treatment.

Key words: Adenosine deaminase – 5'-Nucleotidase – Poly(A)polymerase – Surface immunoglobulin light chain – Chronic lymphocytic leukemia

Introduction

Chronic lymphocytic leukemia (CLL) is a disease of the small mature lymphocytes, usually of the B cell type. Although, morphologically, the cells involved in CLL show relatively homogeneous features, survival of the patients with this disease does not reflect homogeneity. Analysis of surface markers, cytochemical and enzymatic patterns of normal and neoplastic cells have been shown to be relevant to the investigation of human lymphoid leukemias. These studies contributed to the knowledge about the biology of these diseases and to more accurate classification of these neoplasias [12, 21, 32].

Among the enzymes investigated, adenosine deaminase (ADA) and ecto-5'-nucleotidase (5'NT), both purine degradative enzymes, have proven to be of value in defining subsets of lymphoid malignancies [19]. In B cell derived malignancies, the significance of these enzymes has yet to be defined. Nevertheless, it is known that there exists a heterogeneity in the levels of 5'NT activity, possibly related to the differentiation stage of the neoplastic B lymphocytes and that overall, low activities are observed in CLL compared to normal lymphocytes [18, 24, 26]. Adenosine deaminase activities are also reported to be lower in the lymphocytes of patients with CLL compared to normal lymphocytes [35].

Finally, the enzyme poly(A)polymerase, which is responsible for the polyadenylation of mRNA, has been shown to have significantly different levels of activity between acute and chronic leukemias [39]. Furthermore, the heterogeneity observed in the enzyme activity among chronic lymphocytic leukemias was correlated with the aggressiveness of the disease [33].
In the following study we have investigated the activity of the three enzymes in the circulating malignant lymphocytes of 47 CLL patients as well as the expression of surface light immunoglobulin chains which have also been reported to reflect the rate of aggressiveness of the disease [15, 16, 31].

Materials and methods

Materials

[5'-3H] ATP (sp. act. 40–60 Ci/mmol) was obtained from Amersham International Ltd. (Amersham, England). Unlabelled mononucleotides were from Boehringer-Mannheim (Furtwängl, FRG). Adenosine deaminase from calf intestinal mucosa, phenol nitroprusside solution (reagent I) and alkaline hypochlorite solution (reagent II) were from Sigma (St Louis, MO). GF/C flashes were from Whatman Ltd. products (Maidstone, UK). Culture media were from Flow Laboratories, UK. Fetal calf serum (FCS) was a product of Pittrlon (Altona, Australia). The rabbit anti-human IgG or IgA, and the FITC-conjugated swine anti-rabbit immunoglobulin fraction, were purchased from Dako (DK). All other reagents were of analytical grade and were from Sigma.

Methods

Biological material. From 47 newly diagnosed CLL cases, 20 ml of blood were collected in heparinized syringes, before treatment.

Lymphocytes were isolated from peripheral blood by the method of Boyum [2]. Cells collected from the interphase were washed 3 times and in all cases were > 90% viable as determined by trypan blue dye exclusion.

Immunological markers. Typing of lymphocytes in blood samples was performed according to the method of Joly et al. [23] with some modifications. Briefly, 50 μl of the lymphocyte suspension (5 × 10^6 cells) in RPMI 1640 with 1% FCS and 0.01% sodium azide, were incubated for 30 min at 37°C to remove any cytophilic immunoglobulins. The cells were washed once with the same medium and the cell pellet was incubated with 10 μl of rabbit anti-human κ or λ light chains, diluted 1/30 in RPMI 1640 containing 1% FCS and 0.01% sodium azide. After labeling, the cells were washed thrice with cold PBS and a drop of mounting medium (70% glycerol, 30% PBS, pH 0.8) was added to the cell pellet. A drop of the cell suspension was placed on a microscope slide and the cell membrane immunofluorescence was evaluated using a fluorescence microscope. Two hundred lymphocytes were counted and the number of positive cells was expressed on a percentage basis of the lymphocytes counted.

Enzyme tests. Poly(A)polymerase and adenosine deaminase. Assays of the activity of these two enzymes were performed on cell extracts prepared according to the method of Berger and Cooper [1]. Protein was determined as described by Lowry et al. [27].

The assay for determining the activity of poly(A)polymerase measures the incorporation of [3H]-ATP into acid-insoluble product using polyadenylic acid (poly(A)) as an initiator as described earlier [41, 42]. The standard assay mixture (100 μl) contained 200 mM Tris-HCl (pH 8.3), 1 mM MgCl₂, 1 mM [3H]-ATP (10 to 20 cpm/pmol), 4 mM 2-mercaptoethanol, 1 mM (3′OH) poly(A) and 20 μl of the cell extract, diluted to a final concentration of 1 mg of protein per ml, to be assayed. After incubation at 37°C, 20 μl aliquots were spotted at various time intervals on GF/C discs and were processed and counted as described previously [41]. Specific activity is expressed as nmol of radioactive ribonucleotide incorporated per hour per mg of protein.

The adenosine deaminase assay measures the hydrolysis of adenosine to ammonia and inosine. The amount of ammonia released can be quantified using a modification of the Berthelot method [3]. The standard assay mixture contained 2 mM adenosine and cell extract, diluted to a final concentration of 0.25 mg of protein per ml in a final volume of 360 μl PBS. After 30 min or 60 min of incubation at 37°C, 40 μl of reagent I and 40 μl of reagent II were added to 120 μl aliquots of the assay mixture. Final incubation was carried out at 37°C for 15 min. The samples were then brought to a final volume of 0.8 ml by the addition of water and the OD was measured at 630 nm. A standard curve was used to determine the amount of ammonia released. Specific activity is expressed as nmol of ammonia produced per hour per mg of protein.

5'-Nucleotidase. Enzyme activity was measured by colorimetric estimation of NH₃ released from adenosine, the product of 5'NT activity, in the presence of ADA. Using microtiter plates, 2 × 10⁶ intact cells were added to the reaction mixture containing 0.3 mM 5'AMP and 0.04 U/ml ADA in a final volume of 200 μl PBS, according to a method previously described [34]. The OD was measured in an automatic microplate reader (Dynatech TN R 600) with a 630 nm filter. A standard curve was used to determine the amount of ammonia released. Specific activity is expressed as nmol of ammonia produced per hour per 10⁶ cells.

Statistical analysis. Differences between two categories of values were analysed by the t-test of significance. The degree of relationship between two variables has been tested by the method of correlation.

Results

All cases studied were identified as B cell neoplasias. The collective analytical data of the patients are presented in Table 1.

Table 2 summarizes enzyme activity values of 5'NT, ADA and poly(A)polymerase of CLL and normal lymphocytes. Our data show that 5'NT activity values are lower in CLL patients than in normal controls. No significant differences were observed between the mean ADA activity values of lymphocytes from CLL patients and healthy donors. Finally, poly(A)polymerase specific activity values from CLL patients were higher than those from normal peripheral lymphocytes.

The analytical data were further studied in order to identify any correlation existing between the parameters investigated. No correlation was found between the white blood cell count and ADA activity at the time of diagnosis. On the other hand, in two patients who were repeatedly tested and found to show an increase (no. 13) or a decrease (no. 47) in white blood cell count, there was a corresponding variation in lymphocyte ADA specific activity as shown in Fig. 1.