Lactic Dehydrogenase in Normal and Leukemia Lymphocyte Subpopulations: Evidence for the Presence of Abnormal T Cells and B Cells in Chronic Lymphocytic Leukemia

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Lactic dehydrogenase (LDH) was quantitated and the isozyme pattern studied in lymphocyte subpopulations from normal people and patients with chronic lymphocytic leukemia (CLL). Normal T lymphocytes differed from normal B lymphocytes in having greater total LDH activity (597.2 versus 252.1). Total LDH activity in CLL T cells (347.1) was lower than in normal T cells, but not significantly different from normal B cells. Total LDH activity in CLL B cells (124.6) was lower than normal T cells, but not significantly different from normal B cells. Total LDH activity in CLL B cells (124.6) was lower than normal B cells and normal T cells. The isozyme pattern of normal T lymphocytes showed a higher activity in the LDH-1 band (26.7% versus 5.4%) but showed a lower activity in the LDH-5 band (4.3% versus 16.3%) compared to normal B cells. Chronic lymphocytic leukemia T cells could be distinguished from CLL B cells by a high LDH-5 band (22.3% versus 7.6%) and from normal T cells by a high LDH-5 band (22.3% versus 4.3%) and a low LDH-1 band (7.3% versus 26.7%). CLL B cells could be distinguished from normal B cells by a low LDH-5 band (7.6% versus 16.3%). Thus, the LDH isozyme pattern distinguishes normal T lymphocytes from normal B lymphocytes, and normal T and B lymphocytes from CLL T and B lymphocytes.

MATERIALS AND METHODS

Donors

Two hundred milliliters of blood was obtained from 10 healthy males, age 28–52. They had no known diseases and were not taking any medication. Five consecutive, untreated male patients with chronic lymphocytic leukemia, age 58–78, were admitted for study. No selection criteria were used except for a high lymphocyte count and the absence of previous chemotherapy.

The percentage of T lymphocytes were identified by their ability to form rosettes with neuraminidase-treated sheep erythrocytes as previously described. B lymphocytes were quantitated by immunofluorescent membrane staining using fluorescein conjugated polyvalent anti-human immunoglobulin [F(ab')2 fragment] as previously described. By these methods, the percentage of normal T lymphocytes and B lymphocytes was 67.2% ± 8.4% and 9.7% ± 3.5%, respectively.

The 5 patients with CLL had a mean lymphocyte count of 98,000 cells/cu mm (range: 44,000–190,000). The mean percent of immunofluorescent membrane staining cells with anti-human IgM and IgD [F(ab')2 fragment] demonstrated was 93.9% (range: 88%–98%).

Lymphocyte Separation

Peripheral blood mononuclear cells isolated from anticoagulated venous blood by centrifugation at 150 g over a Ficoll-Hypaque gradient. The harvested mononuclear cells were washed four times with HBSS at low speed. This procedure eliminates platelet contamination from the pellet. Each sample contained less than 1000 platelets/ul. The mononuclear cells thus obtained were suspended in RPMI-1640 containing 20% fetal calf serum (FCS) then depleted of monocytes by incubation in tissue culture flasks for...
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1 hr at 37°C in a 5% carbon dioxide atmosphere. The harvested nonadherent cells resulted in less than 2% contaminating monocytes as evaluated by latex ingestion and nonspecific esterase staining of cells.21 Lymphocytes were then separated according to their ability to form rosettes using neuraminidase-treated sheep erythrocytes (SRBC).22 Briefly, mononuclear cells were suspended at a concentration of 5 x 10^6 cells/ml of RPMI-1640-20% FCS with 1% sheep erythrocytes. The rosette-forming cells (ERFC) were separated from non-ERFC on a Ficoll-Hypaque density gradient. The resulting ERFC pellet was resuspended as described and again sedimented over a Ficoll-Hypaque gradient. The resulting ERFC cells were designated as T lymphocytes.22 The T cells were not further purified into Tγ or Tα subpopulations. This procedure gave a cell suspension with less than 2% non-T cells as determined by immunofluorescent membrane staining with polyvalent anti-human IgM and IgD [F(ab')2 fragment]; therefore, these cells fulfill criteria for a B lymphocyte population. The CLI T cell population was purified by SRBC rosetting as described above. Although this population contains B lymphocytes, K cells, and third population was purified into Tγ or Tα subpopulations. This procedure gave a cell concentration at 10,000 cells/ml. Cells were disrupted by sonication at 4°C for greater than 97% immunofluorescent membrane staining with anti-ERFC pellet was resuspended as described and again sedimentation of 5 x 10^6 cells/ml of RPMI-1640-20% FCS with 1% sheep erythrocytes. The rosette-forming cells (ERFC) were separated from non-ERFC on a Ficoll-Hypaque density gradient. The resulting ERFC cells were designated as T lymphocytes.22 The T cells were not further purified into Tγ or Tα subpopulations. This procedure gave a cell suspension with less than 2% non-T cells as determined by immunofluorescent membrane staining with polyvalent anti-human immunoglobulin; greater than 95% cells retained the capacity to form E-rosettes. SRBC were lysed by treatment with tris ammonium chloride;24 this maneuver resulted in no RBC contamination of the T lymphocyte preparation. Viability as assessed by trypan blue exclusion was greater than 97% in all experiments. The non-ERFC in the gradient interphase were collected separately, washed with RPMI-1640 thrice, and SRBC lysed as described. Viability was greater than 95% in all instances. Greater than 96% of non-ERFC demonstrated membrane staining with polyvalent anti-human Ig. Although this population contains B lymphocytes, K cells, and third population cells it will be designated as the normal B lymphocyte population. The CLL B lymphocyte population demonstrated greater than 97% immunofluorescent membrane staining with anti-human IgM and IgD [F(ab')2 fragment]; therefore, these cells fulfill criteria for a B lymphocyte population. The CLI T cell population was purified by SRBC rosetting as described above. Experiments were performed only if greater than 95% of cells retained their ability to form E-rosettes.

Preparation of Cell Extracts

The mononuclear cell preparations were suspended in 50 mM tris-HCl buffer, pH 8.0, and the cell concentration adjusted to 0.5-1.0 x 10^6 cells/ml. Cells were disrupted by sonication at 4°C for 45 sec at 40 KHz with a Biosonic Sonicator (Branwell Scientific, Rochester, N.Y.). The suspension was then clarified by centrifugation at 10,000 g for 10 min at 4°C. The supernae was used for the LDH determinations. Electron microscopy showed solutions to be free of fibrin and membranes.

Lactic Dehydrogenase Assay

LDH isozymes were determined by cellulose acetate electrophoresis for 25 min at 250 V (3.5 - 5.8 A) using a Beckman Microzone with fresh barbital buffer, pH 8.6. The cellulose membranes were stained with nitrotrazolium and phenazine methosulfate solution. The zymograms were scanned on a Beckman Scanning Densitometer (Model R-112) at 570 nm. The values obtained represent the percentual distribution of the LDH fractions.

Total LDH was assayed by a DuPont Automatic Clinical Analyzer (ACAT2) using the methodology described by Gay et al. The change in absorbance at 340 nm due to the appearance of NADH over a 17.07-sec measurement period was used to calculate enzyme activity. Activity was expressed in DuPont International Units per liter per μg protein, (IU/1/μg protein).

Table 1 shows the LDH isozyme pattern in the lymphocyte subpopulations in normal and CLL patients. The isozyme pattern of normal T lymphocytes showed a higher activity in the LDH-1 (26.7% ± 3.1% versus 5.4% ± 1.1%; p = 0.02) band and lower activity in the LDH-5 band, (4.3% ± 1.1% versus 16.3% ± 2.2%; p = 0.04) band than normal B lymphocytes. The percentage of the LDH-2, LDH-3, and LDH-4 bands were similar in normal T and B cells (p ≥ 0.2 for any pair comparison). CLL T lymphocytes showed a higher LDH-5 fraction than CLL B lymphocytes (22.3% ± 3.1% versus 7.6% ± 1.0%; p = 0.01); LDH fractions 1-4 were similar for CLL T and B cells. CLL T lymphocytes could be distinguished from normal T lymphocytes in having a higher LDH-5 band (22.3 ± 3.1% versus 4.3% ± 1.1%; p = 0.01) and a lower LDH-1 band (7.3% ± 1.6% versus 26.7% ± 3.1%; p = 0.02). CLL B cells demonstrated a lower LDH-5 fraction (7.6% ± 1.0% versus 16.3% ± 2.2%; p = 0.04) when compared to normal B cells. Thus, the LDH isozyme pattern of normal T cells was different than normal B cells; similarly, CLL T and CLL B lymphocytes zymograms were distinguishable from their normal counterparts.

The total LDH enzyme activity in normal and CLL lymphocyte populations is shown in Table 1. Normal T lymphocytes had a total LDH activity of 597.2, which was higher than normal B lymphocytes (252.1;
Cells had a higher total LDH activity than CLL B cells and CLL T lymphocytes than normal T lymphocytes in LDH isozyme patterns. Normal T lymphocytes have a higher LDH-1 band with fewer LDH-5 fraction than normal B lymphocytes. These particular data must be interpreted cautiously since our results are based on the assumption of non-ERFC consisting of B cells, K cells, and T lymphocytes. Chronic lymphocytic leukemia T lymphocytes have a different LDH isozyme pattern than CLL B lymphocytes; CLL T cells have a higher LDH-5 fraction than CLL B cells. Our most important finding is that CLL T lymphocytes are completely different from normal T lymphocytes and the majority of circulating lymphocytes in most CLL patients are B cells.

LDH occurs as 5 different isozymes in mammalian tissues. All 5 isozymes consist of 5 different combinations of 2 different polypeptide chains, designated H4 and M4. LDH-1 (H4), predominates in heart muscle, and LDH-5 (M4), predominates in skeletal muscle, liver, and embryonic tissues. The other 3 isozymes, are considered hybrid forms (LDH-2 (H3M), LDH-3 (H2M2), and LDH-4 (HM4)). The biosynthesis of the 2 types of chains and thus, the relative amount of LDH isozyme in a given cell, are under the genetic regulation of 2 alleles controlling LDH-1 and LDH-5 activity. The isozyme patterns of adult human tissues are organ specific, but all fetal tissues have identical patterns with equal activity of the LDH-1 and LDH-5 genes. Although all LDH isozymes catalyze the same reaction, they differ significantly in their Km values for their substrates, particularly for pyruvate as well as their Vmax. LDH-5 (M4) has a low Km and therefore rapidly converts pyruvate to lactate; LDH-1 (H4) has a high Km, converting pyruvate to lactate slowly. These kinetic characteristics yield insight into cellular function. Skeletal muscle and embryonic tissue (LDH-5) utilize glucose anaerobically breaking it down to form lactate during glycolysis. Heart muscle (LDH-1), on the other hand, does not normally form lactate from glucose but oxidizes pyruvate aerobically to carbon dioxide. If the above data can be extrapolated to normal lymphocytes it would appear that T lymphocytes metabolize glucose aerobically; whereas, B lymphocytes (non-ERFC) have a predominantly anaerobic pathway for glucose metabolism. CLL T lymphocytes would behave like normal B lymphocytes in having anaerobic metabolism; whereas, B lymphocytes should have both aerobic and anaerobic capacity for glucose metabolism. This is obviously conjectural and requires verification. Studies by Goldman et al. have shown that most malignant tissue has a predominant LDH-5 isozyme band, perhaps reflecting the anaerobic metabolism of most malignant tissue. Interestingly, CLL T lymphocytes have a predominant LDH-5 band.

Wybran et al. have suggested that the abnormal response of CLL lymphocytes to phytohemagglutinin (PHA) stimulation is due to the presence of normal T lymphocytes contaminating the B cell majority. Our previous data showing the dilution of normal T lymphocytes with mitomycin-C treated (unresponsive) lymphocytes caused no abnormal PHA response refute Wybran’s data. In addition, we have found that purified populations of CLL T lymphocytes and B lymphocytes develop similar, but abnormal PHA growth response.
patterns.\textsuperscript{10} Kay et al.\textsuperscript{11} recently reported imbalances in CLL circulating T cell subpopulations; these investigators found FcγT cells (supposedly suppressor T cells) were markedly increased. Their data support the findings of Faguet\textsuperscript{24} who showed that the delayed PHA response of CLL lymphocytes was due to excessive suppressor T cell activity. Boumsell et al.\textsuperscript{25} have shown that CLL T and B cells share common membrane antigens not shared by normal T and B cells suggesting a close relationship in the state of differentiation between CLL T and B cells. Our data analyzing the LDH patterns in CLL T and B lymphocytes further support the hypothesis that abnormal T cells exist in CLL.\textsuperscript{8,10}

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