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PAPER

A STEREOLOGICAL COMPARISON OF CHRONIC LYMPHOCYTIC LEUKAEMIA CELLS AND NORMAL LYMPHOCYTES

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ABSTRACT

The small lymphocytes which predominate in CLL resemble normal peripheral lymphocytes morphologically. Stereological techniques at the ultrastructural level were used to assess whether quantitative differences could be demonstrated in cell structure. Numerous parameters were estimated - of these only 4: Nuclear volume, Cell surface to volume ratio, Nuclear surface to volume ratio and total cell surface showed statistically significant differences.

INTRODUCTION

In recent years the group of Chronic Lymphocytic Leukaemias has been extensively studied. Clinically it is a heterogenous disease with a variety of presentations and varying prognoses. Immunological studies have demonstrated that the cells belong to a clone of B-cells in the majority of cases. The peripheral blood film is dominated by small, apparently mature lymphocytes. A characteristic feature also is the presence of smear and basket cells the origin of which is unknown. Morphologically these small B-cells closely resemble the majority of circulating lymphocytes in normal peripheral blood, 85% of which are thought to be T-cells.

A stereological study was undertaken to assess possible hidden quantitative differences.

MATERIALS AND METHODS

43 patients presenting with CLL were studied before cytotoxic therapy was administered.

13 normal individuals were studied in comparison.

Methods

Electron microscopy

7-10 mls of venous blood was collected into 5 mls of 6% Dextran (150,000 daltons) containing 500 units of pure heparin. The samples were mixed gently and left at room temperature for one hour to allow the red cells to sediment. The supernatant was then pipetted into approximately four times its own volume of 1.5% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4). The cells were fixed for one hour at room temperature. The sample was then centrifuged at 200 g for five minutes. The supernatant was discarded and the cells were transferred into plastic microtubes and were washed three times using 0.1M cacodylate buffer containing 7% sucrose (pH 7.4). The final pellet of cells at the bottom of the microtube was extracted by first sectioning the base of the tube and exerting gentle pressure with a wooden stick. The final pellet was cut into pieces of tissue (approx. 1 μm^3), post fixed for one hour in 1% osmium tetroxide in (0.1M) phosphate buffer, dehydrated and embedded in Araldite. These sections were cut, mounted on copper grids and stained with 1% uranyl acetate (aqueous) and 1% lead citrate and examined using an AEI 6B or a Corinth electron microscope.

Magnification

The magnification was chosen so that the whole cell was encompassed in a micrograph. For the Corinth this was 7,500 with a final print magnification of 15,000. For the AEI 6B it was 6,300 with a final print magnification of 12,600. A diffraction grating was photographed for each sample to measure magnification.

Stereology

(i) Sampling procedure

Five blocks were obtained from each sample of white blood cells, and one or two sections were obtained from each block. The blocks were obtained from pellets of tissue cut at random following the formation of the whole tissue pellet. The orientation of these blocks on the microtome was random. The final sections obtained were random and representative of the whole tissue. Final random micrographs were taken. The procedure adopted was to select "good" grid squares, i.e. complete, untorn sections with high contrast. Six cell sections lying in the corners of the grids were then photographed. The cells had to belong to the type of cell under study and be clearly identifiable. Nuclear biased sections were therefore taken for all cells.

(ii) Sample size

The sample size required was estimated by using the progressive cummulative means test. A final value within 5% of the final cummulative mean was adopted. This was obtained at approx. 10-15 samples. This sample size was always exceeded for increased precision. About 30 sections were photographed for each cell population studied.

(iii) Measurement

Boundary and area measurements on the electron micrographs of the cells and their contained organelles were carried out using a KONTRON MOP AM03 semi-automatic analyser. Test micrographs were placed on the measuring tablet and a light-weight cursor used to outline each cell and test organelle. Values for the profile length of each test object and the area enclosed within each test boundary and the number of organelles outlined were then available in printed form. The measurements outlined from the MOP printout were the following:-

Area outlined by cursor in mm^2

Perimeter outlined by cursor in mm

Number of cells outlined

Number of nuclei outlined

Number of mitochondria outlined

The appropriate formulae were used (Underwood E. E. 1970): $\frac{S}{V} = \frac{\text{Perimeter}}{\text{Area}} \times \frac{4}{\pi} \times \text{magnification}$

Volume of nucleus was obtained by using the mean intercept area radius = $\sqrt{\frac{3 \times \text{area}}{2\pi}}$

(iv) Correction formulae

The formulae to correct for nuclear bias (Mayhew and Cruz-Orive 1973) were used.

(v) Statistics

The Welch test (Mack 1966) was used to compare mean values.

RESULTS

Table 1 Comparison of 4 parameters for normal and CLL lymphocytes.

	Cell Volume μm^3	Nuclear Volume μm^3	V_V Nucleus %	S/V Cell $\mu m^2\ \mu m^{-3}$
Lympho-cytes	123.3±3.2	52.5±1.7	42	1.41±0.04
CLL	123.2±4.5	58.4±2.1	47	1.26±0.02
Welch Test	NS	$p<0.025$	-	$p<0.005$

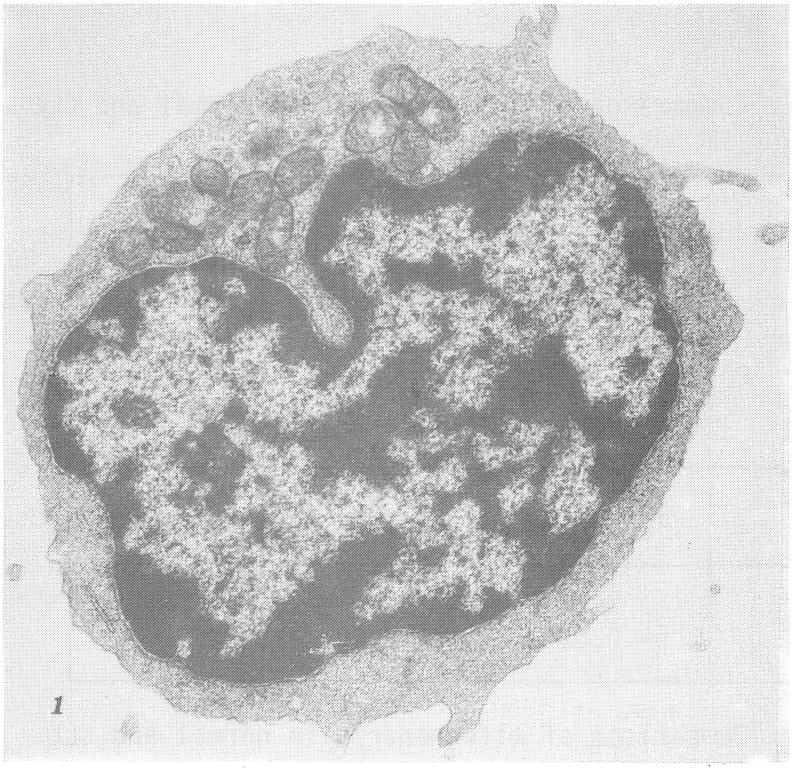
Table 2 Comparison of 3 parameters for normal and CLL lymphocytes

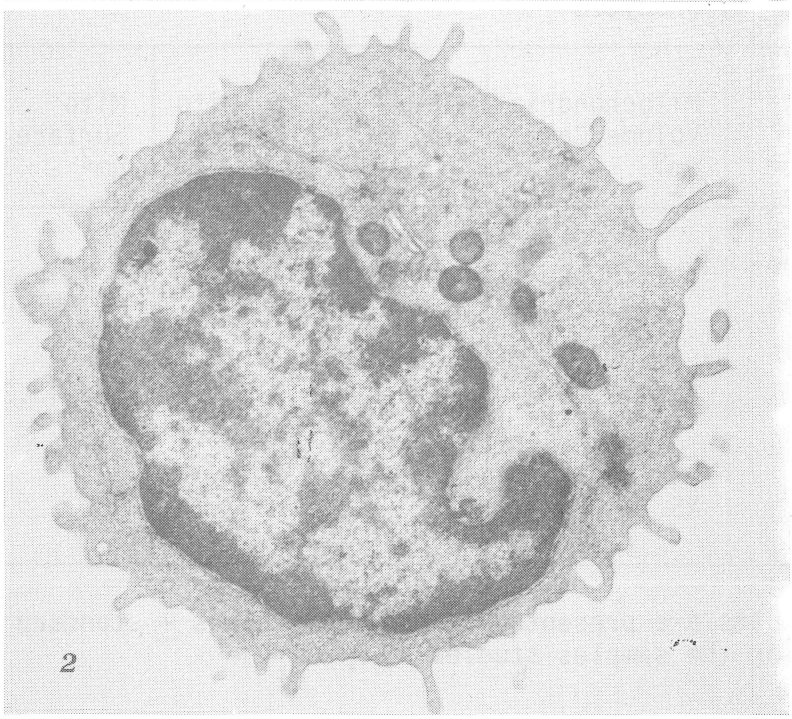
	S/V Nucleus μm^2 μm^{-3}	Cell Surface μm^2	Nuclear Surface μm^2
Lympho-cytes	1.59±0.02	174.2±6.4	82.4±2.2
CLL	1.45±0.02	154.9±5.7	83.0±2.0
Welch Test	$p < 0.025$	$p < 0.025$	NS

Table 3 Comparison of mitochondria in normal and CLL lymphocytes

	Mitochondria Volume/Cell μm^3	Mito S/V μm^2 μm^{-3}	V_V Mito % Cell	Mito Surface μm^2
Lympho-cytes	4.17±0.26	12.5±0.4	3.3	5.18±2.9
CLL	4.70±0.29	11.9±0.3	3.8	55.9±3.3
Welch Test	NS	NS	-	NS

The results are presented as the mean values ± standard error for the samples studied.





Illustrations 1 and 2 show a CLL cell and a normal lymphocyte respectively at a magnification of x 10,000.

DISCUSSION

This is the first quantitative stereological study comparing normal human lymphocytes and CLL cells. The CLL cells represent a clone of B lymphocytes and the values obtained are for the mythical average cell of such a population. Since at the time there was no possibility of separating normal lymphocytes from CLL cells, it is possible that in every patient studied, a few sections from normal lymphocytes were included. It is currently not known what proportion of normal lymphocytes are present at any one time in the peripheral blood of a patient with CLL. Such separation has become possible recently with the use of monoclonal antibodies and further studies are in progress. The normal peripheral blood lymphocytes also are a mixture of T and B and other lymphocytes. Although T cells predominate it cannot be claimed that the values obtained are for T cells.

Only salient characteristics were studied in these cells. Few cell sections contained granules, endoplasmic reticulum either rough or smooth and only a few were sections through the Golgi apparatus. These structures were therefore not quantified. The mitochondria varied in size and shape, it was therefore not possible to obtain an accurate estimation of mitochondrial numbers per cell.

The quantitative results support the qualitative assessment that the cells are similar. Normal lymphocytes appear to have a more irregular surface outline and possibly a more convoluted nucleus. CLL cells show a smoother cell and nuclear outline and a higher nuclear cytoplasmic ratio. It is possible that these results are due to the slightly larger nuclei in CLL cells.

ACKNOWLEDGEMENTS

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