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التوثيق الالكتروني والميكروفيلم

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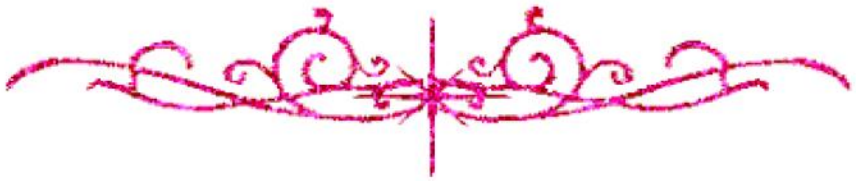

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بالرسالة صفحات

لم ترد بالأصل

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Normal Morphological Variations of Lymphocytes

Thesis for partial fulfilment
of MSc degree
in Clinical Pathology

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The Candidate

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Review Of Literature

Normal Lymphocytes

A. Morphology :

The normal adult human body contains on the order of a trillion $(10)^{12}$ Lymphocytes, most of which appear virtually identical to one another when examined by conventional histologic techniques (Daniel P. Stites. 1994). Regarding the size of cell you may find three different sizes of lymphocytes : small, medium and large lymphocytes. Using the light microscopy to study lymphocyte morphology in the peripheral blood, it was found that :

Small lymphocyte measures from 6-10 µm in diameter. Typically it is round in shape with ovoid or kidney-shaped nucleus that has densely packed nuclear chromatin and almost fill the cell area leaving only a small rim of cytoplasm. The nucleus is stained deeply in Romanowsky stain, while the cytoplasm is stained sky-blue. This basophilia of the cytoplasm is related to RNA content. It is usually free of granules but sometimes, it contains scanty reddish violet azurophilic granules usually 5 to 15 per cell (Smetena et al., 1981).

Large Lymphocyte is about 14 - 20 um in size. It has more abundant cytoplasm. The cytoplasm varies in amount and forms a more extensive area and more basophilic than that of small lymphocyte. The nucleus is more or less rounded. It has a light staining and a fine chromatin network.

Many large lymphocytes have promment nucleoli, particularly those produce by blastic transformation of all lymphocytes under the

influence of mitogens. This large lymphocyte is rarely seen in the peripheral blood but is usually seen in small numbers in the thoracic duct lymph .

The intermediate lymphocyte is found in bone marrow smears or lymphoid tissues. It may be considered as a precursor of small lymphocyte. (Elves, 1972).

*** Transmission Electron Microscopy :**

As visualized by transmission electron microscopy, the circulating lymphocyte measures about 5 µm in diameter. The nucleus has an abundance of electron-dense condensed heterochromatin, a feature characteristic of nonproliferating cells. The nucleoli are round in section, about 1 to 1.5 µm in diameter, and composed of three distinct and concentrically arranged structural units : the central region or agranular zone, the middle fibrillar region ; and the granular zone, which contains intranucleolar chromatin. The lymphocyte's nuclear membrane contains nuclear pores and a perinuclear space .

The cytoplasmic organelles, like the Golgi zone, are poorly developed. The cytoplasm contains free ribosomes, occasional ribosomes clusters, and strands of rough-surfaced endoplasmic reticulum. Centrioles, mitochondria, microtubules (diameter of approximately 0.25 µm), and microfilaments (diameter of about 0.07 µm) are present in the cytoplasm adjacent to the cell membrane. The cytoplasm also contains lysosomes, which are about 0.4 µm in diameter, electron-opaque, and contain classic lysosomal enzymes (e.g. acid phosphatase, ß-glucuronidase, and acid ribonuclease).

The lymphocyte plasma membrane stained with colloidal iron, a marker for membrane sialic acid. Lymphocyte cell membranes and cell coat glycoproteins are shown with other electron-dense markers including phosphotungistic acid, lanthanum colloid and ruthenium red (Douglas SD, 1972).

* Scanning Electron Microscopy :

Scanning electron microscopy provides three-dimensional information (Hayes, 1973). However the resolution achieved with scanning electron microscopy about 0.1 µm is considerably less than that possible with transmission electron microscopy, generally 0.0020-0.0039 µm.

Normal blood lymphocytes, washed and collected onto silver membranes and fixed in glutaraldehyde, have a spherical topography with varying numbers of stubby or finger like microvilli (Polliack A, Lampen N, Clarkson BD, et al, 1973).
T-lymphocytes have smaller numbers of microvilli than B-lymphocytes (Polliack A. Fu SM. Douglas SD. et al.,1974). However the surface morphology of B. lymphocytes is heterogeneous. Many B cells are smooth with few microvilli and thus are indistinguishable from most T lymphocytes (Polliack A. Hammerling V. Lampen N. De Harven E. 1975).

* Phase-Contrast Microscopy :

Active movement of lymphocytes is studied by phase-contrast, or interference-contrast, microscopy. Lymphocytes move slowly with a "hand mirror", appearance. Cytoplasmic spreading does not occur. However, during cell movement a thickening occurs in the cytoplasmic rim (the "Hof" region) that houses most of the cells' organelles, including the Golgi. Lymphocytes from patients with chronic lymphocytic leukaemia have decreased movement (Cohen HJ, 1975).

B. Biochemistry of lymphocytes :

Composition of lymphocytes

Ion and water content :

The resting blood lymphocyte has a mean cell volume of 200 FL and contains 79 ± 1.2% by weight of water (Segal et al., 1981).

The total lymphocyte cation content is 36 fentamol per cell, of which 22 to 28 fentamol per cell is potassium, and 7.9 ± 3.2 fentamol per cell is sodium (Segal et al., 1979). The calcium content of resting lymphocytes has been estimated at 580 to 800 p mol /10^6 cells, and increases several fold following cell activation (Lichtman,et al., 1979).

Organelles :

In large part, the composition and metabolism of long-lived blood T-lymphocytes reflects their resting state. Thus, T-cells have a high nuclear-cytoplasmic ratio, few ribosomes or mitochondria, and scanty endoplasmic reticulum. Glycogen stores are scanty. The DNA content of the resting small lymphocyte (8 pg per cell) is the same amount in other diploid cells (Glen 1987). In contrast, the RNA content average 2.5 pg per cell, yielding as RNA/DNA ratio of approximately 0.32. This value is less than in most other human cells due to the small amount of ribosomal RNA in lymphocytes. The few lysosomes in blood lymphocytes contain several different acid hydrolases including acid phosphatase, ß-glucuronidase, ß-galactosidase, ß-hexosaminidase, α-arabinosidase, α-

galactosidase, α-mannosidase, α-glucosidase, and ß-glucosidase (Pangalis et al., 1978). Acid hydrolase activities are generally higher in T than in non-T-lymphocytes. Lysosomal acid esterase, assayed histochemically with α-naphthyl acetate as substrate, has a characteristic punctate appearance in mature T-lymphocytes (Kulenkampff et al 1977). The granules of cytotoxic T-cells contain a pore-forming proteolytic enzyme, termed perforin, that is released upon activation (Young et al., 1988).

Membrane :

The lymphocyte plasma membrane is composed of equal parts by weight of protein and lipid, and 6 percent by weight of carbohydrate (Crumpton and Snary, 1974). The molar ratio of cholesterol to phospholipid is approximately 0.5 (Goppelt et al., 1986). Phosphatidylcholine is the predominant phospholipid in the lymphocyte plasma membrane but phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and sphingomyelin are also present. Approximately half the membrane fatty acids are saturated. The membrane proteins are usually glycosylated (Johnson and Robinson, 1979).

Enzymes located on the exterior surface of lymphocytes include 5'-nucleotidase, sodium-potassium ATPase, and alkaline phosphatase (Muller-Hermelink, 1974). Levels of 5'-nucleotidase are three to four fold higher in B-lymphocytes than in T-lymphocytes, and increase with the maturation of both cell types. The variably low levels of lymphocytes ecto-5' nucleotidase reported in patients with hypogammaglobulinaemia reflect the immature