دراسة خلايا الليمفا في الأبقار الصحية

الكيمياء بواسطة الميكروسكوب الإلكتروني

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عند دراسة الخلايا المكونة لليف من الأبقار الصحية الكيمياء، وجد أنها تتكون في الغالب من الخلايا الليمفاوية. وهذه الخلايا الليمفاوية يمكن تقسيمها إلى أربعة أنواع:

- خلايا الليمفاوية الصغيرة.
- الخلايا الليمفاوية ذات الحجم المتوسط.
- الخلايا الليمفاوية الكبيرة.
- الخلايا الغامضة.
ULTRASTRUCTURAL STUDY OF LYMPHOCYTE FROM LYMPH OF THE THORACIC DUCT OF CLINICALLY NORMAL CATTLE
(With 5 Figs.)

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SUMMARY

Ultrastructural study of lymph from the thoracic duct of clinically healthy cattle showed that the cellular population of lymph consists mainly of four types of cells of lymphoid series. Small medium sized and large lymphocytes as well as undifferentiated cells. This classification was based upon the diameter of the cells, the nuclear cytoplasmic ratio and the ultrastructural morphology.

INTRODUCTION

There are few reports on ultrastructural study of lymphocytes of lymph nodes and spleen from cattle affected with leukemia (SORANSON and THELEN 1963). UEBORSCHAR (1963-1966) describe certain ultrastructural feature of lymphocytes from haemopotic organs. Also there were few works on the ultrastructural morphology of blood leukocytes of clinically healthy cattle (D.E. BLECHOR MAN; A.N. SMURNOV; B.D. EGROUA 1968).

However analysis of literature revealed that there was no reports concerning the ultrastructural morphology of lymphocyte of lymph of clinically healthy cattle.

This work was designed to study the ultrastructural morphology of lymphocytes from clinically normal cattle.

MATERIAL and METHODS

This work was done at the department of pathology Moscow Veterinary academy. Sex clinically healthy cows were used for this study. All cows were exposed for haematological examination. The amount of leucocytes in the blood ranges from 5,000 to 12,000/mm³ of blood. Directly after surgical opening of the thoracic duct and establishment of artificial fistula, 200 ml of lymph was obtained. Heparin was added as an anticoagulant at a rate of 1 mg/1 ml of lymph. As the lymph contain a certain amount of red blood cells. Distilled water was added to lymph in ratio of 1:2 per volume, then mixing and NaCl 4% solution was added in ratio 1:1 per volume to prevent swelling and rupture of lymphocytes. This lead to haemolysis of erythrocytes. The lymph was centrifugated at 1000 R/m for 5 m. The supernatant fluid was removed and the precipitate was then fixed in 2.5% solution of glutaraldehyde for 25-30 m. The disc of cell was then divided into small pellets, washed in phosphate buffer with pH 7.4 several times. The pellets were post fixed in osmic acid, then washed with phosphate buffer 3-5 times. Dehydration was done in serial dilution of ethyl alcohol and acetone. The pellets were embedded in gelatine capsule contain the mixture of epion and araldehyde. Ultrathin
sections were obtained on ultratome L K B. 8802 A using glass knife. The ultrathin section was stained by urinal acetate and potassium citrate (method of REYNOLDS 1963).

RESULTS and DISCUSSION

Electron microscopic examination of lymph from thoracic duct of clinically healthy cattle showed that the cellular population of lymph consisted mainly of cells of lymphoid series with few amount of granulocytes, leucocytes and red blood corpuscles. The per centage was 99.4% lymphoid cells 0.2% neutrophils, 0.3% eosinophiles and 0.1% Monocytes. The majority of cell were those of lymphoid series. According to the size of the cells, ratio between the size of the nucleus and cytoplasm and the ultrastructural morphology, four types of cells could be distinguished.

Small lymphocyte:

This was the predominant type of lymphocyte observed. The diameter was 5-6 U km. The ratio between the nucleus and cytoplasm was high ranging from 5:1 or more. The nucleus was round or oval or may be indented, usually centrally situated, coarse chromatine located at the periphery in either isolated masses or in continuous band. This masses or bands sometimes extend towards the middle part of the nucleus. The nucleolus was absent. The fine chromatine was usually situated in the center and somtimes extend to the periphery. Somtimes the coarse chromatine was diffusely distributed all over the nucleus with small amount of fine chromatine. The perinuclear area was distinct. Nuclear pores were not prominent. The cytoplasm was represented by narrow band, surrounding the nucleus. The organelles in the cytoplasm were very scanty (Fig. 1). Only free ribosomes could be demonstrated in the cytoplasm of this cells. Mitochondria, smooth, rough endoplasmic reticulum and Golgi apparatus were not observed. Vacuoles of pinocytosis were observed.

Medium sized lymphocyte:

The diameter of this cell ranging from 6-8 U. This cell was observed in fewer amount than the small lymphocyte. The ratio between the size of the nucleus and cytoplasm was lesser than those of small lymphocyte (average 3.5:1). The nucleus was not centrally situated. The coarse chromatine was arranged in the form of clamps which usually observed at the periphery of the nucleus and somtimes extend towards the middle portion. The coarse chromatine occupied a small part of the nucleus. The nucleus was well developed. The perinuclear space was clear, while the nuclear pores were not observed. The cytoplasm appeared as a relatively broad band surrounding the nucleus, being broadest at the end where the cytoplasmic organelles were condensed (Fig. 2). The cytoplasm of this cell was relatively rich in organelles. Free ribosomes were relatively abundant. Mitochondria with condensed crusts were also found. Its number was ranging from 3-5 and were rounded in shape. Golgi apparatus was seen they were represented by electron clear small rounded or oval vacuoles. smooth and rough endoplasmic reticulum also not observed.

Large lymphocyte:

The amount of these cells was fewer than the preceeding two types. The diameter ranges from 8-10 U with nucleo-cytoplasmic ratio of 1.5:1. The nucleus in this cell was eccentirically situated being rounded or oval in shape. The coarse chromatine was either diffusely distributed in the nucleus or arranged in clumps at the periphery of the nucleus. Fine chromatine was abundant in amount and found usually in the middle part of the nucleus. The perinuclear area was
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not distinct, while the nuclear pores were clear. The cytoplasm was more or less abundant and was very rich in organelles (Fig. 3). The number of mitochondria ranges from 8-10. They were oval rounded, elongated or polyhedral. The mitochondrial crest were usually of electron clear type. Golgi apparatus was well developed. It was represented by electron clear vacuoles or tubules arranged side by side. Few ill developed granular endoplasmic reticulum were also seen. The amount of free ribosome was larger if compared with the preceeding types. Sometimes ribosomes unite to form rosette shape polysomes. Vacuoles of pinocytosis were few.

Undifferentiated or blast cells:

The diameter of this cell was about 10 U or larger. The nucleocytoplasmic ratio was 1.9:1. The nucleous was oval or rounded occupying a large part of the cell. Coarse chromatine was arranged on the periphery near the nuclear membrane in form of small or large masses. The amount of fine chromatine was larger than that of coarse one and occupying the largest area of the nucleous being concentrated in the middle part. (Fig. 4). The nucleolus were constantly seen. The paranuclear space was clear and the nuclear pores were distinct. The cytoplasm was of mild electron density. It contains scanty amount of free ribosomes which may coalesce to form rosette shape polysomes. The amount of mitochondria (3-5) with electron dense crests were present. Golgi apparatus was not well developed. It was represented by few numbers of small electron clear vacuoles, multivesicular bodies were also observed. Single ill developed endoplasmic reticulum were detected while granular endoplasmic reticulum were absent.

Our classification of lymphocyte according to diameter was in agreement with the classification given by Cimoin and AGACIN (1968). These authors classify lymphocytes from the blood of healthy and leucotic cattle into small, medium sized and large lymphocytes using light microscope. FEDROV (1967) stated that blood lymphocytes from healthy person according to the diameter and ultrastructural morphology could be divided into 4 types. Large lymphocytes, small light & dark lymphocytes and lymphoplasmocytes. He added that small light lymphocytes constitute 75% of all types. According to our result small lymphocytes could not be distinguished into dark and light type, lympho-plasmocytes were not observed in our material. However small lymphocytes constitute the main type of lymphoid cells of the lymph from thoracic duct of clinically healthy cattle as in healthy human blood. The ultrastructural morphology of large & small lymphocytes described by this author are inagreement with the ultrastructural morphology encountered in our material with exception that the stated that lymphocyte exhibit 1-2 mitochondria in its cytoplasm. This statement was given by BLEKMAN B.E. et al. 1968. A general description of fine structure of lymphocyte from spleen and lymph node of clinically healthy cattle was given by FEDROV, N.A. et al. 1973, but they did not classify lymphocytes into different types.

From our result we can concluded that the cellular popualtion of lymph obtained from the thoracic duct of clinically healthy cattle consists mainly of four types of cells of lymphoid series. Small lymphocyte, medium sized lymphocyte, large lymphocyte and undifferentiated cells. The predominant type was the small lymphocyte. The ultrastructural morphology was described in details. This result may be of great help in comparative study of lymphocyte of lymph of the thoracic duct of cattle with lympholeukosis and lymphosarcoma in the preceeding work.

REFERENCES


DESCRIPTION OF FIGURES

Fig. (1 a,b): Ultrathin section of small lymphocyte with a cytoplasm cantion only few ribosomes.
Fig. (2): Ultrathin section of medium sized lymphocyte with cytoplasmic organelles condensed at the broadest end.
Fig. (3): Ultrathin section of large lymphocyte with cytoplasm rich in organelles.
Fig. (4): Ultrathin section of undifferentiated cell with large amount of fine chromatine occupying the middle part of the nucleus.

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