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ISOLATION OF C TYPE VIRUS PARTICLES FROM
LEUKEMIC AND LYMPHOCYTOTIC CATTLE

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INTRODUCTION:

It is well established that viruses are the cause of avian leukosis, murine leukemia and feline leukemia. The status of the etiology of other animal leukemias is less well understood. It is a widely proposed hypothesis that leukemia of cattle is also caused by viral agents. This view is supported by the results of epidemiologic, 1,2,3,6,7,11,17,18 transmission studies^{8,14,15,19,20} and the reports of the isolation of virus and virus-like particles from leukemic cases.^{4,5,9,12,13,16,21} There is not adequate evidence at present to prove the viral etiology of bovine leukemia since the biological activity of the isolated virus agents has not been reported and transmission studies have been inconclusive.

The objective of this preliminary paper is to report on the isolation of C-type virus particles from leukemic cattle and cattle with a persistent lymphocytosis (lymphocytotic) utilizing cell culture and electron microscopy. Lymphocytotic animals were included in this study because of the evidence that these animals are in the prodromal stage of bovine leukemia.^{2,11,14,18}

MATERIALS AND METHODS:

SOURCE ANIMALS - The seven leukemic cows, five lymphocytotic cows and five normal control cows used in the study were selected on the basis of previously defined criteria.¹⁰

LYMPHOCYTE CELL CULTURE - The procedure for separation of lymphocytes from blood was similar to that reported by Joel et al.¹⁰ Blood was obtained by jugular venapuncture, defibrinated and the lymphocytes were separated by the silicone method. The lymphocytes thus separated were suspended in Eagle's MEM containing 25% bovine fetal serum and antibiotics (300 units of penicillin, 150 ug of dihydrostreptomycin, 100 ug of neomycin and 5 ug of fungizone per ml.) to a concentration of one million cells per ml. one hundred ml. of this suspension was distributed into each tissue culture flask* and 2 ml. of phytohemagglutinin M** (PHA) was added. Cells were harvested for electron microscopic studies following 72 hours of incubation at 37°C.

ELECTRON MICROSCOPY - Lymphocyte cultures were pelleted by centrifugation. The cell pellets were fixed with 2% gluteraldehyde, postfixed with 2% osmium tetroxide, dehydrated with graded alcohol and then embedded in maraglass. Thin sections were stained with lead citrate and then with uranyl acetate and examined under electron microscope RCA EMU 3.***

*Falcon Plastic Co., 5500 West 83rd Street, Los Angeles, California.

**Difco Company, Detroit, Michigan.

***RCA Company, Camden, New Jersey.

RESULTS AND SUMMARY:

Virus particles were observed in 72 hour phytohemagglutinin stimulated lymphocyte cultures. Lymphocyte cultures from cases having high lymphocyte counts of an immature type yielded more virus particles. The virus particles were C-type with central nucleoid surrounded by a single or occasionally a double membrane (Fig. 1). They were extracellular and 65-85 mu in diameter. The virus particles were usually observed in groups located closely adjacent to lymphocytes (Fig. 2,3,4), often in the vicinity of the cytoplasmic processes of the lymphocytes. There were buddings of virus particles from the plasma membrane into the cytoplasmic vacuoles (Fig. 5) and also on the cell surface of the lymphocytes (Fig. 1).

The C-type virus particles were isolated from five of seven leukemic cows and five of five lymphocytotic cows. One of the five lymphocytotic cows developed the tumorous phase of leukemia four months after she was studied initially. At this time she was again studied and virus particles were found. All five normal control cows studied were negative for any virus particle.

It is premature to assess the biological significance of C-type particles in leukemic and lymphocytotic cattle but the association of C type virus particles to murine and feline leukemia and avian leukosis

strongly suggests that these viral particles may be of etiologic significance. The potential significance of these viral particles is further supported by the failure to isolate viral particles in defined normal control cattle.

Fig. 1 Electron micrograph of PHA stimulated culture of lymphocytes from lymphocytotic cow. The virus particles appear to have a double membrane. Budding of a virus particle at the cell surface is seen (arrow). X 100,000.

Fig. 2 Electron micrograph of PHA stimulated culture of lymphocytes from leukemic cow. Note the presence of virus particles adjacent to a lymphocyte. X 30,000.

Fig. 3. Electron micrograph of PHA stimulated culture of lymphocytes from leukemic cow. The virus particles are in a group and located outside of the lymphocyte. X 100,000.

Fig. 4. Electron micrograph of PHA stimulated culture of lymphocytes from leukemic cow. The virus particles are in the vicinity of cytoplasmic processes. X 100,000

Fig. 5. Electron micrograph of PHA stimulated culture of lymphocytes from lymphocytotic cow. Budding of virus particles from the plasma membrane into the vacuoles in the cytoplasm. Matured virus particle (arrow) are located outside the lymphocyte. X 40,000

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