Preliminary Report on Virus-like Particles in Canine Leukemia and Derived Cell Cultures


Introduction

Lombard et al. (9) reported the transmissibility of canine mastocytoma with mast cell leukemia to dogs, with attempts to transmit to other species. In addition, with the electron microscope they demonstrated "viral-like" particles within the cytoplasm of the cells. In the present study 2 dogs with reticulum cell leukemia were obtained for light and electron microscopic evaluation. Fluid and cells from the pleural cavity in one case leukemia were obtained for light and electron microscopic study. In the other case, a large, rounded or reniform, loosely-textured nucleus, prominent nucleoli, and a moderate amount of cytoplasm. In this report, the term "reticulum cell" designates a large cell with a large, rounded or reniform, loosely-textured nucleus, prominent nucleoli, and a moderate amount of cytoplasm.

The purposes of this study were (a) to determine whether virus-like particles could be identified in the lymphatic tissues of these leukemic dogs, and (b) to examine the canine cell cultures and media for viral particles.

Materials and Methods

Canine Leukemia. A spontaneous reticulum cell neoplasm with a leukemic pattern of involvement developed in a male German shepherd (D-1), age 3 years and in a male Weimaraner (D-6), age 8 years. In both animals, there was enlargement of the liver, spleen, and lymph nodes; in D-1h tere were plaques and nodules in the pleura, and pleural and peritoneal effusion.

Microscopically the liver, spleen, lymph nodes, bone marrow, pleura and lungs, and most other organs of the body were diffusely infiltrated with reticulum cells (Fig. 1). The peripheral blood smear showed moderate numbers of blast cells.

Cell Culture Technique. Ten ml of cell fluid were aspirated from the pleural cavity of D-1, mixed with an equal volume of Eagle's minimal essential medium plus 10% calf serum and dispensed in 2.0-ml amounts to screw cap tubes. This cell culture was maintained in 2 separate laboratories to ensure its survival. A lymph node, obtained from D-6, was minced, trypsinized, and suspended in Medium 199 plus 10% bovine fetal serum. Both media contained 100 units of penicillin and 1 µg of amphotericin B/ml. The cells were grown as monolayers and dispersed by trypsinization.

Mycoplasma Isolation. Throat swabs were made with cotton-tipped applicators which were placed in 2.0 ml of Tryptose-phosphate Broth (Difco) containing 200 units/ml of penicillin and 1 µg/ml of amphotericin B. The throat culture broth and the fluid aspirated from the pleural cavity were inoculated on Petri dishes (0.1 ml/specimen) containing Chanock's medium (2) and incubated at 37°C in an atmosphere of 5% CO₂ in air.

The mycoplasma was recovered from both the throat and the pleural fluid. It was also recovered from the early passages of the cell culture, where it was deliberately eliminated with 200 µg/ml of streptomycin.

The mycoplasma was identified as Mycoplasma canis by Dr. R. B. Purcell (NIH, Bethesda, Md.), using a fermentation inhibition test (14).

Light and Electron Microscopic Technique. Tissues for light microscopy were fixed in 10% buffered formalin, embedded in paraffin, and sections stained with hematoxylin and eosin. Smears for cytologic studies, obtained from peripheral blood, bone marrow, and imprints of organs were air dried and stained by the Wright-Giemsa method.

The tissues for electron microscopy were fixed in 4% buffered glutaraldehyde solution, pH 7.2 (12), and postfixed in osmium tetroxide. The tissues were dehydrated in successive changes of 70, 80, 95, and 100% ethanol and embedded in an Epon-Araldite mixture. The mycoplasma pellets were prepared for electron microscopy using a technic similar to the one described by Anderson and Barile (1). Cells and fluid from the canine cell cultures were centrifuged and prepared according to a technic described by Dalton and Moloney (5), sectioned with a Porter-Blum (MT-1) or Cambridge microtome using glass knives, and

Summary

A light and electron microscopic study was made of lymphatic tissues from 2 dogs with reticulum cell leukemia. Virus-like particles were found in both dogs. Cultures of leukemic cells were established from both dogs and 1 culture contained numerous C type virus-like particles. Mycoplasma canis was recovered from the pleural fluid and pleural fluid cell culture of 1 dog. Comparison of this mycoplasma with the virus-like particles showed the former to be more pleomorphic and much larger. M. canis was eliminated from the cell culture and the particles seen in subsequent passages were assumed to be viral rather than a form of mycoplasma.

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stained with uranyl acetate (13) and lead citrate (11). For negative staining a small drop of the resuspended centrifuged media was placed on a carboned grid. After 30 see the material was blotted and the grid inverted on the surface of a 2% solution of potassium phosphotungstate, pH 5, for 1 min. All of the material was examined and photographed with an RCA EMU-3G electron microscope.

Spleen, lymph nodes, and pleural cells from D-1 and spleen and lymph nodes from D-6 were examined by electron microscopy. In sectioned tissue, the number of particles observed was correlated with the area of tissue examined. The area was determined by the number of nuclear sections observed. A low particle frequency of +1 was used to indicate that 362 to 2400 nuclear sections were observed before finding a particle, whereas +4 indicated that less than 120 nuclear sections were observed before finding a particle (3).

Results

Virus-like particles were located in the cytoplasm and budding from the plasma membrane of both leukemic dogs (Figs. 2–7). The particles were 80–100 m with a nucleoid of 50–60 m. There was, in most cases a thin outer coat but no outer unit membrane structure around these particles. The particle frequency of both animals (D-1, D-6) was estimated as +1. The cells associated with these particles were characterized as reticulum cells or lymphocytes. The virus-like particles were somewhat similar to the C type particles described in certain murine leukemias (4, 6), but it could not be determined if they were similar to those described by Lombard et al. (9) in canine mast cell leukemia.

An area containing a minimum of 4800 nuclear sections from the spleen and lymph nodes of 2 adult nonleukemic dogs was examined and no virus-like particles were found.

CANINE CELL CULTURE. Examinations were made of the cell cultures derived from both the leukemic dogs (D-1, D-6). The cultured cells from the pleural fluid of Dog D-1 were characterized as epithelioid and those from the lymph node of Dog D-6 as fibroblastic. The dog’s pleural cells from which this epithelioid cell culture was derived showed virus-like particles (Fig. 4) and so did the lymph node used for the fibroblastic cell culture (Figs. 3, 6). The presently existing epithelioid cell culture was examined periodically between the 7th and 44th passages and many virus-like particles were found, not only within vacuoles, but also in an extracellular position (Figs. 8-10). These particles measured 80–100 m, with a nucleoid of 50 m and there was a particle frequency of +4. No virus-like particles were found in the fibroblastic canine cell culture from D-6 which stopped growing at the 9th passage.

M. canis was isolated from the epithelioid cell culture and original pleural fluid of Dog D-1. An ultrastructural comparison was made between the centrifuged pellets of the cell culture media and a pure culture of M. canis. The negatively stained particles in the cell culture media were virus-like and had tail-like structures. The heads of the particles measured approximately 100 m in diameter and were reasonably consistent in size and shape (Fig. 11). In contrast, the mycoplasma were pleomorphic, and at least 10 times larger than the virus-like particles (Figs. 12, 13). Mycoplasma were not obtained from the other dog (D-6) or from the fibroblastic cell culture derived from this animal.

Sections from the centrifuged pellets from the 7th through the 44th passages of the epithelioid cell culture were examined and many virus-like particles, measuring approximately 100 m, were seen in each pellet (Fig. 14). These virus-like particles were morphologically similar to those obtained from murine leukemias (4, 6). The sections from centrifuged pellets of the isolated M. canis demonstrated structures that were dissimilar to the virus-like particles (Figs. 13, 14). M. canis was eliminated from the cell culture and later passages have not yielded mycoplasma when cultured. This therefore decreases the possibility that the virus-like particles seen in later passages were mycoplasma.

Cell free filtrates and cell suspensions from the epithelioid cell culture free of M. canis have been inoculated into puppies and axenic mice. The experimental procedures have been designed to observe surviving animals for a minimum of 1 year. Presently, these studies are in an early stage of observation, but, preliminary examination of 1 dog, inoculated with a filtrate, showed virus-like particles. In a series of experiments using infant axenic mice, a leukemia has developed which is of the same cell type as the leukemia in the original dog (D-1). Also, numerous virus-like particles were found in these mice. These results will be reported in detail upon the completion of the experiments.

Discussion

The M. canis originally isolated from the leukemic dog (D-1) was examined with the electron microscope and found to be morphologically different from the virus-like particles. This mycoplasma was shown to be morphologically similar to M. hominis as described by Anderson and Barile (1). The retention of virus-like particles after M. canis was eliminated, indicates the organisms are separate entities. This organism may have been part of this dog’s normal microbial flora since throat swab cultures from 30 nonleukemic dogs have all yielded mycoplasma. Failure to recover mycoplasma from the other leukemic dog (D-6) may be explained by inadequate sampling. Although one must be cautious in implicating mycoplasma as a leukemogenic agent, the obvious presence of this organism in the original leukemic dog cannot be ignored (10).

Virus-like particles seen in the original canine leukemic tissues were somewhat similar to those observed in murine leukemias, human leukemic lymph nodes (7, 8), and in axenic mice inoculated with human leukemic material (3). This suggests that a morphologically similar particle, present in these species, may be associated with leukemia.

A comparison was made between leukemic tissues from leukemic dogs and similar normal tissues from nonleukemic dogs. Although no virus-like particles were observed in the spleens and lymph nodes from the nonleukemic animals, this does not exclude the possibility that these animals could be carrying a small number of virus-like particles. In addition, more healthy as well as chronically debilitated animals must be examined for virus-like particles and mycoplasma before these results can be fully evaluated.

Cells from the pleural fluid of 1 leukemic dog (D-1) and cells from the lymph node of 2nd leukemic dog (D-6) were established in cell culture. Examination of the epithelioid cells and super-
natant fluids (D-1) demonstrated the presence of large numbers of C type particles similar to those seen in the original pleural cells. The lack of particles in the fibroblastic cell culture may indicate there were no particles present in the initial cells or that fibroblasts would not support the replication of the virus-like particles. This study is presently being extended to examine, in greater detail, the possible viral etiology of canine leukemia.

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References


Fig. 1. Reticulum cell infiltration in liver of dog, D-1. The major portion of the field contains leukemic cells with uniform, large loosely-textured nuclei and large nucleoli. A few hepatic cells are present in the left portion of the photomicrograph. × 450.
Fig. 2. Virus-like particles within the spleen of a leukemic dog (D-6). Two of the particles appear to be budding from endoplasmic reticulum. × 102,500.

Fig. 3. Virus-like particles budding from the plasma membrane within the lymph node of a leukemic dog (D-6). × 102,500.

Fig. 4. Virus-like particles budding within 1 of the pleural cells of a leukemic dog (D-1). It was from these pleural cells that the epithelioid cell culture was derived. × 140,000.

Fig. 5. Virus-like particles forming on the surface of a cell from the lymph node of a leukemic dog (D-1). × 140,000.

Fig. 6. Virus-like particles within the matrix of a cell from lymph node of leukemic dog (D-6). × 140,000.
FIG. 7. Section from leukemic canine spleen (D-6) shows intracytoplasmic particles (arrows). \( \times 60,720 \).
Fig. 8. Section of virus particles from canine cell line derived from leukemic animal (D-1). These particles were morphologically similar to the mature murine C type particle. × 145,600.

Fig. 9. A virus particle obtained from a section of another culture of the canine cell lines derived from leukemic cells (D-1) is similar to the immature murine C type particle. × 106,600.

Fig. 10. Section from canine cell culture derived from pleural cells of leukemic dog (D-1) indicates the representative cell type present in this culture. × 11,900.
FIG. 11. Virus-like particles from the tissue culture media negatively stained appear to have tails and, again, are similar to murine particles. × 48,000.

FIG. 12. *Mycoplasma canis* particles obtained from a broth culture. These particles were isolated from 1 of the leukemic dogs (D-1). Note the pleomorphism and translucent quality of the structures. Negatively stained. × 48,000.

FIG. 13. Section of pellet from broth culture of *Mycoplasma canis* isolated from leukemic dog (D-1). Shows homogenous structures with variations in size. Compare with section of virus-like particles in Fig. 14. × 48,000.
FIG. 14. Section of pellet obtained from the canine cell culture shows large numbers of virus-like particles similar to murine C type particles. $\times 137,200$. 
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