Electron Microscopic Observations of Leukemia in Animals and in Man

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It took many years to realize that cancer is a disease that affects every living creature, no matter what its place may be in the scale of evolution. In spite of impressive progress in our knowledge of the biology of cancer, it is somewhat disappointing to reflect that many practitioners of medicine and scientists of the present day, whether dealing with fish, birds, animals, or man, apparently fail to realize that there is an undoubted unity of phenomena that lead to cancer in animals and man. There is hardly any doubt that more knowledge has been gained about cancer by studying birds and animals than by exploring the disease in man. This is not to say that studies on human cancer are premature, but to emphasize that such studies require full knowledge of, and proper understanding of the technics employed in, experiments on animal cancer. It appears at the present time that only if one is armed with knowledge of the results of studies on animal cancer and what they have contributed to our understanding of cancer as a general biologic phenomenon can he make a rational approach to the study of the origin of cancer in man; even then, this approach may not be successful at first. Studies on animal cancer may at least help, if not speed up, the final aim of cancer research—that is, knowledge of the cause, specific cure, and successful prevention of human cancer.

During the past 14 or 15 years viruses have been found to be involved in an ever increasing number of tumors in animals of various species. This finding has led to the questions whether viruses are also involved in at least some types of cancer in man and how a search for human cancer virus or viruses should be attempted (3).

Among a number of approaches to this search for a hypothetical human cancer virus, electron microscopy has played a leading part in our laboratory. Electron microscopy was chosen because it has contributed greatly to our knowledge of the ultrastructure of normal, malignant, and virus-infected cells. It has also contributed to knowledge of the structure of viruses (4).

The required knowledge and background were therefore available as a starting point for an election microscope study of animal tumors at first of known, then of suspected, viral origin and for a study of the viruses involved in their development. It may be stated at this point that electron microscopy has contributed greatly to our knowledge of the structure of viruses—of those inducing either infection or cancer. This structure as seen in the electron microscope agrees with the structure of viruses shown by crystallography and X-ray diffraction. It must be pointed out that, in spite of the impressive advance in knowledge of the structure of cells made possible by electron microscopy, no qualitative difference has been observed between normal and malignant cells. Quantitative differences, however, and the presence of virus particles distinguish the cancer cells. This latter property was soon found to be shared by many normal cells in apparently healthy individuals—animals or men. This at first somewhat perplexing observation was eventually found to have a meaning that is probably of far-reaching importance in animal cancer and possibly in cancer of man.

After our original observation of characteristic virus particles in organs of mice with spontaneous leukemia, electron microscope studies were carried out in collaboration with my associates, C. E. Grey, F. Padgett, and Dr. L. Gross, on organs of mice and rats with leukemia induced by cell-free preparations of leukemic organs of mice. Virus particles similar to those present in leukemic organs of mice with spontaneous or induced leukemia were observed in the bone marrow, spleen, thymus gland, and lymph nodes of leukemic rats. The mode of development and the relationship of these particles to cellular constituents appear to be the same in mouse and rat leukemia, whether lymphatic, myeloid, or stem cell. Of particular interest are the cylindrical or filamentous forms, which appear to be a stage in the development of the characteristic virus particles and are found in the megakaryocytes of the bone marrow of leukemic mice and rats (8) (Figs. 1–9).

These virus particles have been found in great numbers, long before the appearance of clinical symptoms of leukemia, in the milk of mice from strains with a high incidence of leukemia and in the milk of mice given inoculations of leukemia virus (Figs. 10, 11).

These virus particles have also been observed in the ovaries of mature, healthy mice and, in small numbers, in the bone marrow, spleen, and thymus gland of embryos of mice from a strain with a high incidence of leukemia (6). They have also been found in similar organs of young (1-day- to 28-week-old), apparently healthy mice of the same strain in gradually increasing numbers (Figs. 14–20). This raises interesting points for discussion on the mode of transmission of leukemia virus in mice with spontaneous and induced leukemia. Future studies will decide whether the leukemia virus is transmitted by the transplacental or the transovarian route. There appears to be little doubt that leukemia in mice and rats may be classified as a "predetermined disease."

Thus characteristic tumor virus particles appear to be more widely distributed than originally suspected. This may be an important point to bear in mind in the electron

1 Supported in part by Grant E-94-F from the American Cancer Society and by USPHS Research Grants CA-04140-06 and CA-05831-02 from the National Cancer Institute.
microscope study of tissues or fluids of apparently cancer-free patients.

It should be added that electron microscope studies have also demonstrated morphologically similar virus particles in all chicken tumors, Rous sarcoma, and leukemia, lymphomatosis, erythroblastosis, and myeloblastosis (4). This finding is similar to those in mouse and rat leukemia, in which morphologically similar particles known to be the causative agent are found in all forms, whether lymphocytic, monocytic, stem cell, or other type of leukemia.

Characteristic virus particles similar to myxovirus particles have been found in the blood of leukemic mice and rats by Dalton and his associates (2) and in the blood of leukemic chickens by Beard and his associates (1).

As shown by the negative staining technic with potassium phosphotungstate, the virus particles of mice and rats, whether obtained from leukemic organs, milk, or blood of leukemic animals, appear to be morphologically similar to the particles of myxoviruses, i.e., influenza, mumps, etc. (Figs. 12, 13).

With this background of information available, electron microscope studies have been carried out by us, in collaboration with Drs. C. D. Howe, C. C. Shullenberger and J. A. Sykes and Mr. C. E. Grey, on biopsy material from lymph nodes of patients with different types of leukemia and malignant lymphoma and on such material after tissue-culture passage. The original findings of these studies were reported some time ago (7) and have since been extended to a larger number of cases.

Electron microscope studies on sections of lymph nodes from patients with lymphatic or myeloid leukemia or with malignant lymphoma in the majority of cases have demonstrated virus particles similar in size, mode of development (such as budding of plasma membrane and cylindrical forms), and relationship with cellular constituents to particles in mouse and rat leukemia. However, morphologic similarity does not indicate identity or the part played by these particles in human leukemia (Figs. 21–28).

It should be emphasized that these findings do not indicate that these virus particles are the causative agent of human leukemia, since they may be a passenger virus. Nevertheless, it is of interest that in all cases and in different types of human leukemia they appear morphologically similar.

As already mentioned, virus particles have been recovered from the milk of mice in the absence of overt disease, i.e., leukemia (6). This finding, the recovery of cancer virus particles from blood of rats and mice (2), and the observations on material from leukemic patients (7) have resulted in a study of blood plasma from leukemic children, carried out in cooperation with Dr. H. G. Taylor, W. Sutow, D. A. Dreyer, J. A. Sykes, and Mr. C. E. Grey, and in a similar study of blood plasma from leukemic patients in collaboration with Drs. C. D. Howe and C. C. Shullenberger (5).

Structures resembling virus particles and similar to myxovirus particles have been observed in the blood of leukemic children and of leukemic adults in the majority of the cases examined (Figs. 29–32) (7). They have also been found in the blood of some apparently healthy control individuals.

The results of these studies have led us to initiate a study, in cooperation with Dr. P. Condit of the Oklahoma Medical Research Foundation, Oklahoma City, that could be described as “microepidemiology” of leukemia, which is known to occur in certain families. An electron microscope study of blood plasma from identical twins, one of whom is leukemic, and their immediate family has revealed particles resembling virus particles (myoviruses) in the blood of the leukemic twin and in that of his mother, but not in the blood of his father, his twin brother, or his other 3 brothers. The significance, if any, of this observation may perhaps be elucidated by a long-term study of blood taken at regular intervals from all members of the family and by an attempt at correlation of the presence or absence of such structures in the blood with clinical findings. These studies are now being carried out.

It may be of interest to mention another observation, carried out in cooperation with Dr. P. Condit, on the blood plasma of a very interesting patient. This patient, a girl, was first seen at the age of 8 years with a diagnosis of acute lymphoblastic leukemia. Dr. Condit relates that she was treated with Delta-core and aminopterin initially, and later with azaserine and G-mercaptopurine. Complete remission occurred and has persisted to the present time. At the time of the electron microscope examination of her blood, the patient had survived for 7¾ years with normal growth and development, reaching the age of 16 years. In spite of careful and exhaustive examination of her blood, no structures resembling virus particles could be found. However, her blood plasma was not examined at the time of her illness, and a comparison with blood examination at that time is therefore lacking.

These studies appear to indicate the necessity of periodic examination of patients admitted with a diagnosis of leukemia. It is especially advisable, since it is now possible to use as little as 0.5—1.0 ml of blood plasma for the purpose of electron microscopic examination. By such studies we may obtain information about the correlation between the virus particles and the state of progression of the disease, especially in members of families with a history of leukemia. In our opinion, such studies are justifiable and require extensive application. Should we fail to obtain any correlation between the presence of the described structures in blood and the presence or the cause of leukemia, these studies will help to eliminate these structures or particles from our minds, if not from the electron microscope.

Such studies may also gain in significance by correlation with serologic studies in which viral and anti-viral antibody spectra are determined throughout life and correlated with the electron microscope findings and clinical data.

No conclusions can yet be drawn as to the relationship between the findings in animals and those in man. Nevertheless, the results appear to be sufficiently encouraging to continue this type of study of human leukemia as well as other types of cancer.

Although the question of viral origin of human cancer still remains in the realm of hypotheses, the solution of this hypothesis—it is our earnest belief—will come through intimate collaboration between practitioners of medicine and those confined to laboratories, in which not only the
specialist but also the general practitioner and family physician have a great deal to offer and will contribute substantially to the final answer.

REFERENCES


Fig. 1.—Appearance of virus particles in cytoplasmic inclusions in the mesenteric lymph node of an AKR strain mouse with spontaneous lymphatic leukemia. X 60,000.

Fig. 2.—Tubular or filamentous structures budding from the membrane of a cytoplasmic vacuole in a cell of a mesenteric lymph node of an AKR strain mouse with spontaneous lymphatic leukemia. Formation of immature or doughnut-type particles by budding or segmentation of the cylindrical structures may be seen. X 60,000.

Fig. 3.—Appearance of virus particles in a mesenteric lymph node of a mouse (C3Hf strain) with induced lymphatic leukemia by passage A (Gross) virus. X 70,000.

Fig. 4.—Appearance of virus particles in cytoplasmic inclusions and vacuoles of a cell in the thymus gland of a mouse (C3Hf strain) with passage A (Gross), virus-induced lymphatic leukemia. X 45,000.
Fig. 5.—Appearance of virus particles in the spleen of a mouse (C57Bl strain) with induced myeloid leukemia. \( \times \) 40,000.

Fig. 6.—Part of the cytoplasm of a megakaryocyte in the bone marrow of a mouse with lymphatic leukemia induced by cell-free preparation of leukemic organs of a rat with passage A (Gross) virus leukemia. Cylindrical or tubular structures (arrows) and vesicular or doughnut-type particles are present. \( \times \) 70,000.

Fig. 7.—Virus particles in the intercellular space of a mesenteric lymph node of a rat with lymphatic leukemia induced by passage A (Gross) virus. \( \times \) 60,000.

Fig. 8.—Budding phenomenon, leading to virus particle formation, of a plasma membrane of a megakaryocyte in the bone marrow of a rat with lymphatic leukemia induced by passage A (Gross) virus. \( \times \) 135,000.
Fig. 9.—Cylindrical or tubular structures (arrows) in a megalakaryocyte of the bone marrow of a rat with lymphatic leukemia induced by passage A (Gross) virus. Segmentation and budding of the structures may be seen. \( \times 75,000 \).

Fig. 10.—Appearance of virus particles in a high-speed, centrifugal pellet of defatted and decaseinated milk from a young, healthy AKR strain mouse long before the development of clinical symptoms of leukemia. \( \times 56,000 \).

Fig. 11.—Part of Fig. 10 at higher magnification. Double membrane surrounding virus particles may be seen. \( \times 135,000 \).

Fig. 12.—Virus particles, stained with potassium phosphotungstate, present in a high-speed centrifugal pellet of defatted and decaseinated milk from a young, healthy mouse of a strain (C58) with a high incidence of lymphatic leukemia. Some particles show tail-like structures. \( \times 75,000 \).
FIG. 13.—Appearance of a virus particle, stained with potassium phosphotungstate, present in a cell-free preparation of leukemic tissues from a mouse (AKR strain) with spontaneous lymphatic leukemia. × 165,000.

FIG. 14.—Part of the cytoplasm of a cell in the thymus gland of an embryo of AKR strain, which has a high incidence of lymphatic leukemia. An immature or doughnut-type particle (arrow) is present. × 70,000.

FIG. 15.—Part of the cytoplasm of a cell in the thymus gland of a 4-day-old, healthy AKR strain mouse. Virus particles are present in a cytoplasmic structure (arrows). × 37,000.

FIG. 16.—Part of the cytoplasm of a cell in the spleen of a 10-day-old, healthy AKR strain mouse. A virus particle (arrow) is present in an osmiophilic body. × 53,000.
Fig. 17.—Part of the cytoplasm of a megakaryocyte in the spleen of a 10-day-old, healthy AKR strain mouse. Occasional nature virus particles (arrow) are present in the specific granules. X 68,000.

Fig. 18.—Part of the cytoplasm of a megakaryocyte in the bone marrow of 14-day-old, healthy AKR strain mouse. Occasional virus particles (arrow) are present in the specific granules. Budding of the membrane of a specific granule (upper part of the picture) can also be seen. X 60,000.

Fig. 19.—Specific granule with a virus particle (arrow) present in a megakaryocyte of the bone marrow of a 6-week-old, healthy AKR strain mouse. X 180,000.

Fig. 20.—Virus particle in a cytoplasmic inclusion (arrows) of a cell in the mesenteric lymph node of a 10-week-old, healthy AKR strain mouse. X 112,000.
FIG. 21.—Budding of the plasma membrane (arrows) of a cell in the lymph node of a patient with acute lymphatic leukemia. × 180,000.

FIG. 22.—A doughnut-type particle almost separated from the plasma membrane of a cell in the lymph node of a patient with acute lymphatic leukemia. × 90,000.

FIG. 23.—Virus particles, mature and doughnut-type, at the plasma membrane of a cell in the lymph node of a patient with acute lymphatic leukemia. × 55,000.

FIG. 24.—Virus particles in cytoplasmic vacuoles in the lymph node of a patient with acute lymphatic leukemia. × 68,000.
FIG. 25.—Virus particles, some with tail-like structures (arrow), in the intercellular space of a lymph node from a patient with acute leukemia. × 100,000.

FIG. 26.—Virus particles in the intercellular space of a lymph node from a patient with lymphoma. × 90,000.

FIG. 27.—Particles with tail-like structures (arrows) in a cytoplasmic vacuole of a lymph node from a patient with acute leukemia. × 180,000.

FIG. 28.—Cylindrical structure (arrow) in a cytoplasmic vacuole of a lymph node from a patient with acute leukemia. × 60,000.
FIG. 29.—Virus-like particles in the blood plasma of a patient with acute leukemia. × 60,000.
FIG. 30.—Virus-like particle at higher magnification. Eccentrically located denser part may be nucleoid. × 165,000.
FIG. 31, 32.—Appearance of virus-like particles in the blood plasma of a leukemic child. × 240,000.
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