Editorial

Viral Etiology of Leukemia and Lymphomas

By Ludwik Gross

CONSIDERABLE PROGRESS has been accomplished during the past 15 years in the study of the nature of leukemia. It seems more and more apparent that the various forms of leukemia and lymphomas, not only in chickens, mice, rats, and other animal species, but also in humans, are caused by filterable and transmissible viruses.

Our information on the nature of this disease in humans is still very fragmentary. It should be stressed that our means of determining the cause of an obscure disease are limited; however, if a disease can be transmitted from host to host by experimental inoculation of filtrates, it can be assumed that such a disease is caused by a virus. It has long been known that chicken leukemia is of viral origin; this was the first animal species in which leukemia was successfully transmitted by means of filtrates. For a long time it was thought that the same disease in the mouse and other mammals might be of a different nature, since all previous attempts to transmit leukemia in mice by filtrates had failed. The turning point was marked in 1951, when transmission of mouse leukemia by filtrates succeeded, using newborn mice for inoculation of cell-free extracts. This observation was accepted with certain reservations not only because it was at variance with generally accepted concepts, but also because cell-free transmission of mouse leukemia could be reproduced at first only with some difficulty. Only later on was it realized that leukemic mouse donors serving as source for harvesting of the virus very often yielded only small quantities of infective virus particles. This was true particularly for mice that developed leukemia spontaneously, or which developed leukemia following x-ray irradiation. However, in the course of successive isolations of the virus from spontaneous mouse leukemias, a few animals yielded relatively potent extracts. One of these filtrates, containing a potent leukemogenic virus, was passed serially from mouse to mouse; the potency of this virus strain gradually increased, and became stabilized. This mouse leukemia virus, designated “passage A,” and comparable perhaps to Pasteur’s “fixed virus” of rabies, since it has a known and predictable pathogenic potential, was found to induce a wide variety of leukemias and lymphomas, following inoculation into suckling mice of several different inbred strains. These forms included not only lymphatic leukemia, myeloid leukemia, stem-cell leukemia, etc., but also lymphosarcomas, reticulum-cell sarcomas, and Hodgkin’s-like lesions. The same virus was found also to be pathogenic for newborn rats, inducing the same variety of leukemias and lymphomas in that species of animals.
Once the experimental conditions were determined under which the leukemic virus could be isolated from mice, and the necessity of using newborn hosts for bio-assay was emphasized, it became a routine procedure to isolate the leukemic virus from different mouse tissues. Numerous individual isolations of the mouse leukemia virus have been reported from various laboratories. It became quite apparent that the mouse leukemia virus is widely prevalent not only in tissues of leukemic mice, but also in tissues of healthy animals, as well as in a variety of transplanted mouse carcinomas and sarcomas. It is quite possible and, in fact, probable that all the usual forms of leukemia and lymphomas observed to develop spontaneously in mice, or induced in animals of that species by radiation, hormones, or carcinogenic chemicals, are caused by a single virus. A large majority of leukemia virus strains isolated from mouse tissues in different laboratories may actually represent isolations from different sources of the same virus, or at best one of its close variants. These virus strains induce in mice and rats the same disease. They also have the same physical properties and the same morphology. The differentiation of such strains would require serologic studies; these, however, encounter considerable difficulties since the leukemia virus of mice has a rather limited antigenic potential. For that reason a suitable and potent immune serum is not available at this time.

The properties of the mouse leukemia virus have been studied, and the virus itself has been visualized with the aid of the electron microscope. The mouse leukemia virus is a small spherical particle about 100 nm in diameter with one or two outer membranes, with or without an electron-dense centrally located nucleoid. In certain phases of development the virus particle consists of a doughnut-like shell surrounded by one or two membranes (immature virus), whereas in other phases of development the virus particle contains also an electron-dense nucleoid (mature, infective virus). The virus particles replicate not only in leukemic cells, but also in a variety of apparently normal cells of the hematopoietic system, particularly in megakaryocytes and also in many different blood cells of both the lymphatic and myeloid series. When scanning with the electron microscope, under high magnification, ultrathin sections of organs of leukemic mice and rats, virus particles can be detected without difficulty in many organs such as in bone marrow, thymus, spleen, liver, lymph nodes, etc. The particles are formed by budding from the cytoplasm either into cell vacuoles or, more frequently, from the cell membranes into the intracellular spaces. Within the cytoplasm, numerous particles can be found in vacuoles; they can also be found in large quantities in intercellular spaces.

Considerable information has now been accumulated on the nature of mouse leukemia and on the properties and epidemiology of the mouse leukemia virus. In mice this virus is transmitted from one generation to another directly through the embryos. This was determined by bio-assay in the early studies; more recently, spherical particles similar in morphology to the mouse leukemia virus were also found in organs of embryos removed from young, healthy female mice of the high-leukemia Ak strain.
Under certain, rather limited, experimental conditions the mouse leukemia virus can also be transmitted through the milk, particularly when the nursing female is either suffering from leukemia, or is at the point of developing this disease. Recent electron microscopic studies revealed the presence of large quantities of virus particles in the milk of female mice of the high-leukemia Ak strain, and also in milk of C3H(f) female mice inoculated with the passage A leukemia virus. Examination of ultrathin sections of mammary glands of pregnant C3H(f) female mice injected with the virus revealed budding of virus particles from epithelial mammary gland cells into the intercellular spaces, and presence of fully formed virus particles in the milk ducts of such mice.

Unlike the majority of common infectious diseases, the mouse leukemia virus is only exceptionally transmitted from one host to another within the same generation. Transmission by contact occurs, but only among newborn mice, and then only to a very limited degree. Exposure of susceptible newborn mice to virus-injected brothers and sisters raised in the same nests resulted in a low incidence of leukemia not exceeding 7 per cent.

Experiments thus far performed suggest that the mouse leukemia virus is pathogenic only for mice and rats, and not for other species. Similar species limitations appear to be valid for other leukemic viruses, such as the virus of chicken leukemia. Thus it seems that the leukemia virus has a narrow host range, and that there is therefore no danger for humans of contracting this disease from virus-carrying chickens, cattle, or other species. It should also be stressed that the mouse leukemia virus is thermodurable, and that it can be readily inactivated by heating to only 50 C. for 1/2 hour. If leukemic viruses in other species also have a similar sensitivity to heat, it would follow that they could be readily inactivated in milk by standard methods of pasteurization.

There is no direct experimental evidence to prove that leukemia and lymphomas in humans are of viral etiology. The evidence, if any, is purely circumstantial, and is based on the comparison of human disease with that observed in mice, and other animal species, in which the viral etiology has been demonstrated. Direct experimental evidence is now available suggesting that the various forms of leukemia and lymphomas occurring in chickens, mice, and rats are caused by viruses. This evidence is based on fundamental studies dealing with experimental transmission of leukemia and lymphomas in these animal species by cell-free extracts. Preliminary results recently reported suggest that certain forms of leukemia in dogs, cats, and cattle can be also transmitted by filtrates, and are therefore caused by viruses. More recently, transmission of a form of human leukemia occurring in African children, into monkeys, has also been reported. This recent observation would add at least one form of human leukemia to the list of leukemias and lymphomas demonstrated to be caused by transmissible viruses; this preliminary report, however, must await further confirmation and clarification; the question was raised whether the disease observed in the inoculated monkeys was induced by the inoculum, and actually represented transmission of
the lymphoma, or whether it was a form of simian bone disease, observed in monkeys kept in captivity ("cage disease"), and unrelated to the injected extracts.\textsuperscript{24}

The principal properties of leukemia viruses in several animal species, particularly in chickens and mice, have been studied and described in detail. The development of precise electron microscopes with high resolution power, the improvement in embedding of specimens, and in the technic of ultrathin sections, and the introduction of staining methods in electron microscopy, led gradually to the recognition and identification of virus-like particles of characteristic morphology. Examination of ultrathin sections revealed not only the size and shape of the virus, but also the presence and number of outer membranes, their width and spacing, the presence, size and location of the electron-dense nucleoid, etc. These morphologic details were of considerable importance in identification of the virus particles found in tissues of leukemic chickens, mice, rats, and other animal species,\textsuperscript{3} and more recently also in human patients suffering from leukemia and lymphomas.\textsuperscript{25,26}

In conclusion, it is now quite apparent that not only in animals, but also in humans, the various forms of leukemia and lymphomas are caused by filterable viruses. In all probability these viruses have a narrow host range; it also appears that, like many other oncogenic viruses,\textsuperscript{3} the leukemia-inducing viruses are transmitted in their respective host species from one generation to another, and only very exceptionally among newborn hosts within the same generation. One of the most prominent features of these viruses is the fact that they remain in most instances latent. Under normal life conditions they cause no apparent harm to their hosts; however, when triggered into action, they may change from harmless parasites into pathogenic agents, causing then rapid multiplication of cells, and inducing the development of various forms of leukemia and lymphomas in their carrier-hosts.

REFERENCES

VIRAL ETIOLOGY OF LEUKEMIA AND LYMPHOMAS

Monograph no. 4, 1960, p. 7.


Ludwik Gross, M.D., F.A.C.P., Chief, Cancer Research Unit, Veterans Administration Hospital, Bronx, N. Y.
Editorial: Viral Etiology of Leukemia and Lymphomas

LUDWIK GROSS