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Received Feb. 12, 1969. P.S.E.B.M., 1969, Vol. 131.

A Study Correlating Virus Particle Frequency with Disease Progression in a Transmitted Murine Leukemia* (33974)

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(Introduced by S. J. Wilson)

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In a detailed pathogenesis study, Potter and Richter (1) described and charted the development and spread of leukemia induced by cellular transmission in the C58 strain of mouse. Since this study most of the published works have involved virus-induced (2–4) and radiation-induced (5) pathogenesis studies of leukemia. The pathogenesis studies by Siegler and Rich (2, 3) reported the light microscopic findings with special emphasis on the preneoplastic changes in the thymus.

The presence of virus particles in organs of leukemic mice have been reported in detail (6, 7). Buffet *et al.* (4) reported the isolation of leukemic virus from organs of Ha/ICR Swiss mice as early as 1 week following inoculation with cell free filtrate of leukemic tissues, therefore indicating the presence of virus particles prior to the development of leukemia. Rich and Johns (8), in a recent publication, reported a similar level of virions in the thymus of their controls when compared with the virus-inoculated ICR/Ha Swiss mice. This implied that the increase in virions was not causally related to the development of the leukemia that followed.

It was our purpose to study relationships between the appearance of virus and the development of murine leukemia following the inoculation of leukemic tissue homogenates.

Materials and Methods. The mice used in this study were randomly inbred axenic CFW mice designated as CFW_w (9). The axenic CFW_w mice were used because of (a) their low indicence of spontaneous leukemic ($<0.1\%$), and (b) the apparent low number of virus particles (9).

A CFW_w mouse (second passage) originally derived from a case of spontaneous lymphocytic leukemia in a conventional CFW_w mouse was selected as donor material for this study. The spleen, lymph node, and thymus were examined by electron microscopy and both intracytoplasmic type A and type C particles were present. A total of twenty-two 2–4-day-old mice were inoculated intraperitoneally with 0.1 ml of a 20% homogenate of spleen, thymus, lymph nodes, and liver from the donor animal. To eliminate the possibility of mice dying with leukemia before they could be examined, the moribund mice were sacrificed early. This selective process left some animals that did not develop leukemia until the end of the experiment. The inoculated mice were autopsied at approximately

* Supported in part by USPHS Grants CA 04888-06, CA-10427-01, 5 T1 A1 137-06, and 1 SO1 Fr-05373, and the University of Kansas Institutional Grant No. 65R-1497-7.

TABLE I. Correlation of Particle Frequency by Electron Microscopy (EM) and Presence or Absence of Leukemia by Light Microscopy (LM).

After inoculation of leukemic cells (days)	Case no.	EM[b]	LM[b]
2	W-3027	0	—
	W-3028	+1	—
4	W-3029	0	—
	W-3030	+1	—
6	W-3031	+2	—
	W-3032	+1	—
8	W-3033	+3	+[a]
	W-3034	+3	+[a]
10	W-3035	+3	+[a]
	W-3036	+4	+
12	W-3037	+4	+
	W-3038	+1	—
14	W-3039	+2	—
	W-3040	+4	+
16	W-3041	+4	+
	W-3042	+4	+
18	W-3043	+4	+
20	W-3045	+4	+
23	W-3047	+4	+
27	W-3049	+4	+
35	W-3050	0	—
36	W-3054	+1	—

[a] Beginning leukemia.

[b] + = disseminated leukemia; — = no leukemia.

2-day intervals and the study terminated on day 36. Autopsies were performed on all animals. Gross and microscopic examinations were made of thymus, lymph nodes, spleen, and most other organs.

For light microscopy, tissues were fixed in 10% buffered formalin, embedded in paraffin, and stained with hemotoxylin and eosin. Fresh air-dried blood smears, bone marrow smears, and organ imprints for cytologic studies were stained by the Wright–Giemsa technique.

Tissues for electron microscopy were fixed in osmium tetroxide or a 2.5% glutaraldehyde solution, embedded in an epon-araldite mixture, and examined with an RCA EMU-3G electron microscope. The particle distribution was determined by counting the number of nuclear sections in an area (9) taken from the spleen, thymus, and lymph nodes of each anima. Quantitation was based on a 0 to +4 scale, (0=no particles and +4= numerous particles). The virus particles were designated according to the classification of oncogenic RNA viruses (10). The particle frequency was correlated with the presence or absence of leukemia.

Results. Pathology. Animals examined the first 6 days after inoculation (6 animals) showed no leukemic cellular infiltration. The majority, 12/14 animals examined from days 8 to 27 had leukemic infiltration in various organs and tissues. Animals examined on days 35 and 36 (2 animals) showed no evidence of leukemia. In the leukemic animals, generally, lymph nodes throughout the body, spleen, and liver were enlarged whereas the thymus was not enlarged. Microscopically, lymph nodes, spleen, liver, thymus, bone marrow, and most other organs were diffusely infiltrated by leukemic cells; and the blood was leukemic. The leukemia in all mice was lymphocytic, and the cell was a large lymphocyte which, in imprint preparations, measured 12–16 μ in diameter as compared to 8–10 μ for normal lymphocytes.

Particle frequency and leukemia: Table I and Fig. 1 summarize the relationship between particle frequency and leukemic involvement of the animals. With particle frequency and leukemic involvement of the ani-

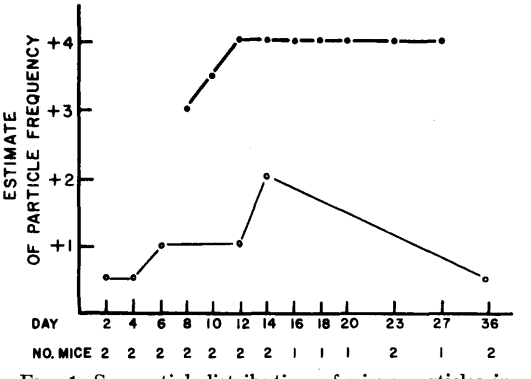

FIG. 1. Sequential distribution of virus particles in the tissues of mice inoculated with leukemic material: (●), the high particle frequency in the inoculated mice which developed leukemia; (○), the low particle frequency in the inoculated mice with no evidence of leukemia. A particle frequency of +3 or +4 was correlated with leukemia.

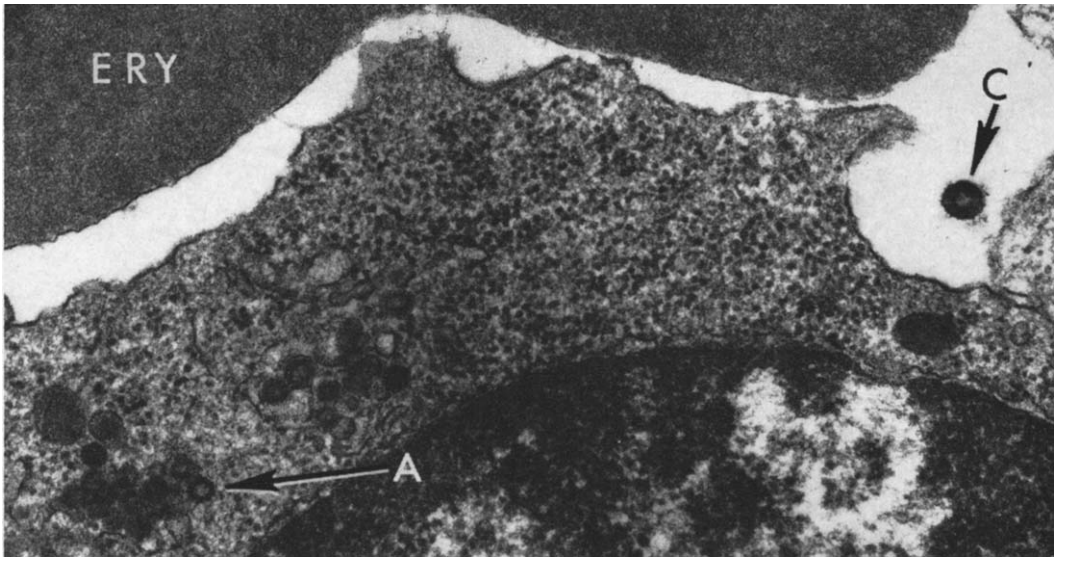

FIG. 2. Intracytoplasmic A particles (A) and an extracellular C type virus particle (C) in the capillary of a mesenteric lymph node from animal W-3037 with lymphocytic leukemia, inoculated 12 days earlier. The A particles are located in a circulating lymphocyte adjacent to an erythrocyte (ERY); ×47,800.

mals. With a particle frequency of 0 to +2 the animals were not leukemic. With a particle frequency of +3 all the animals showed early development of leukemia, i.e., leukemic infiltration confined to structures adjacent to the peritoneum and retroperitoneal tissues. With a particle frequence of +4, all the animals had generalized leukemic infiltration.

A virus particle was found in animal W-3028 on day 2 after inoculation. This particle (C type) was seen in the thymus but none was found in the spleen or lymph nodes and this animal was not leukemic on histological examination. Beginning at day 8 the moribund mice were removed at least every 2 days and the majority showed leukemia through day 27. All of the leukemic animals demonstrated large numbers (+4) of both intracytoplasmic A and C type virus particles in an approximately equal distribution in the thymus, spleen, and lymph nodes. An interesting finding was the presence of a free C type particle and lymphocyte with intracytoplasmic A type particles in the lumen of a blood capillary in a mesenteric lymph node (Fig. 2). It was found that C type particles in the thymus were present in extracellular areas around lymphocytes and not associated exclusively within the thymic epithelial cells. This is in contrast to the study made on the uninoculated nonleukemic conventional CFW_w mice in which the C type particles were localized predominantly within the cisternae of thymic epithelial cells (11).

Since there was not a 100% incidence of leukemia during the 36-day study the remaining mice were then sacrificed at intervals following day 27 and both were nonleukemic. In these two mice the particle frequency was low (Fig. 1, Table I).

Discussion. The results indicate that an estimate of +3 or +4 particle frequency occurred only in inoculated mice with leukemia and provide evidence of association between high virus quantity and disease.

There are several possibilities that may explain this relationship between the virus frequency and disease. Because it has been reported that uninoculated axenic mice have virus (12, 13) one possibility is that the virus observed in the recipients may have no relationship to the inoculated virus. However, in our studies on 13 uninoculated CFW_w axenic mice (9), we found no virus particles. This is a distinct possibility because virus was observed in mice with no evidence of

leukemia during the entire observation period. The lack of observable oncogenic effects may indicate that these viruses, though apparently able to replicate, were either defective or that a larger quantity of virus may be necessary to produce oncogenic effects. The third possibility is that the donor cells and viruses multiply and disseminate in the recipient. The susceptibility of these mice to the leukemogenic effects of virus (data from our laboratory, A. A. Werder) would indicate that some virus could be replicating in the recipient. However, because of the relatively short interval of time required for the development of leukemia the high number of viruses found is most likely due to proliferation and dissemination of the virus-producing inoculated cells.

Summary. A technique was applied to correlate the quantity of virus particles with (a) time following inoculation of leukemic cells, and (b) the presence or absence of leukemia. The CFW_w axenic mice were inoculated with murine leukemic cells containing virus. The inoculated animals were examined every 2 days for quantity and type of virus particles and for evidence of leukemia. Results demonstrated the presence of particles 2 days after inoculation. The first evidence of leukemic infiltration by light microscopy occurred on day 8 after inoculation. All mice that were diagnosed as leukemic by light microscopy showed a high particle frequency (+3 to +4). The high particle numbers closely correlated with the presence of leukemia and not necessarily with the time after inoculation. It is suggested that most of the virus found in the inoculated animals are derived from the donor cells.

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Received Feb. 12, 1969. P.S.E.B.M., 1969, Vol. 131.