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Pathogenesis of Mouse Viral Leukemia.* (26368)

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Virus-induced thymic lymphoma, with secondary generalized leukemia, is anatomically identical with the spontaneous disease in the high leukemia AK strain. The striking difference between them is the considerably shorter latency of virus-induced disease.

A study of the pathogenesis of viral-induced leukemia was undertaken by sacrificing mice at various intervals after viral infection, examining the organs, notably the thymus, for leukemic changes and assaying the thymus for malignant lymphocytes by grafting thymic fragments on young, highly susceptible, isologous mice.

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The site and origin of the leukemic cell of spontaneous thymic lymphoma in the AK strain was determined 16 years ago(1) by a similar procedure. The observations on viral-induced leukemia, here reported, suggest the essential identity of its pathogenesis with that of spontaneous leukemia, the only difference being shorter latency for neoplastic transformation of thymic lymphocytes following viral infection at neonatal age.

Materials and methods. Virus. Four different filtrates of Gross's passage virus A(2), which has been carried in our laboratory since 1957, were used. Preparation and storage of filtrates have been described(3).

Mice. AK/Z mice (F_1 hybrid from AKR/Jax females and C3H/f/Bi/G/L[1] males) were used. Newborn litters (less than one week of age) were injected subcutaneously with 0.025 to 0.05 cc of filtrate. Alternate control litters were either not injected or injected subcutaneously with 0.05 cc of phosphate buffer at less than one day of age. Littermates (males and females) were caged together; offspring of virus-injected females were discarded. *Intercurrent infections* were treated by addition of veterinary terramycin to drinking water.

Experimental procedures. Some virus-injected and control mice were observed until natural death; others were sacrificed at regular intervals. Sections were taken of numerous organs; special attention was given to thymus. Kidney and thyroid were routinely searched for microscopic polyoma lesions. Sections of virus-injected and control mice were studied simultaneously, as unknowns.

Transplantation assays were performed with thymuses taken at various intervals by grafts made intramuscularly in thighs of isologous mice. When only one recipient was used, one-half of each lobe of thymus was grafted in both thighs; when 2 recipients were used, one-half of each lobe was grafted on each recipient; the other halves of each thymic lobe were processed for microscopic study. When 4 recipients were used, about one-third of each lobe was processed for section; the remaining two-thirds of each lobe was cut into 4 equal pieces, and each recipient was grafted with one piece of each lobe in each thigh.

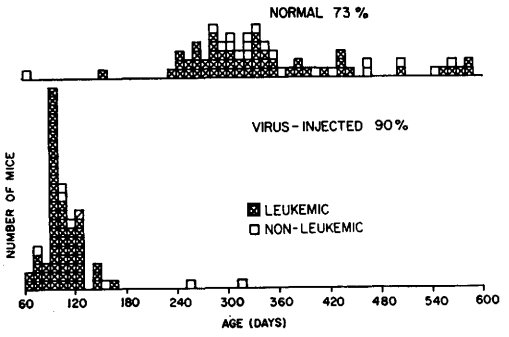


FIG. 1. Incidence and time of occurrence of leukemia in virus-inj. and normal uninj. AK/Z mice.

TABLE I. Microscopic Findings in Thymuses of Virus-Injected AK/Z Mice.

Age of thymus (days)	No. studied	Microscopic findings*
13-20	6	N N N N N N
21-28	7	±? N N N N N N
29-35	8	±? N N N N N atr atr
36-42	14	±? N N N N N N N N N N N N atr
43-49	6	±? N? N? N N atr
50-56	10	+ + N N N N N N N N
57-63	7	+ + ±? N? N? N N
64-70	5	+ + ±? ±? atr
71-77	9	+ + + + + + + + +

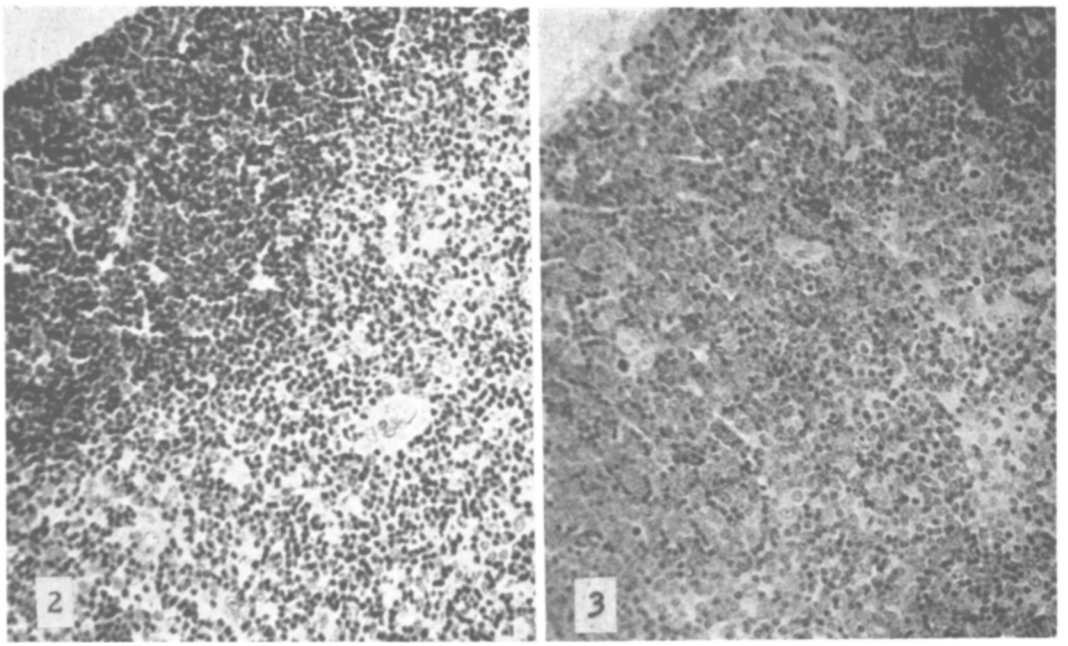
* Each letter or symbol refers to one mouse; N = normal, ±? = questionable preleukemic change, + = distinct leukemic change, atr = atrophy, N? = probably normal.

Results. Incidence and time of occurrence of leukemia in 67 virus-injected and 73 control mice are shown in Fig. 1. Virus-injected mice had a higher incidence of leukemia (90%) than normal mice (73%). However, leukemia occurred much earlier in virus-injected than in control mice. The latency period was slightly shorter in females than in males in both groups, as has been noted in spontaneous leukemias of several strains.

Morphogenesis (Table I). During the first few days of life the cortical area of the thymus of both injected and control mice was wide containing many immature medium to large lymphocytes; the medulla was wide with abundant reticular-epithelial cells. Maturation to the adult pattern, in which the predominant cell of the deeply-staining cortex is the small lymphocyte, was not retarded by virus.

Earliest morphologic change in virus-injected mice was noted at about 40 days, becoming more definite after 50 days. At this time, disseminated small foci of medium to large lymphocytes appeared either subcortically or somewhat deeper in the thymus. Subsequently, these areas enlarged, became confluent (Fig. 3, 5 *vs.* Fig. 2, 4), and by 70 days began to spread beyond the capsule. This sequence of events occurred with remarkable uniformity.

The thymus of 5 animals was involuted without any evidence of leukemia. These in-



From the thymus of AK/Z mice

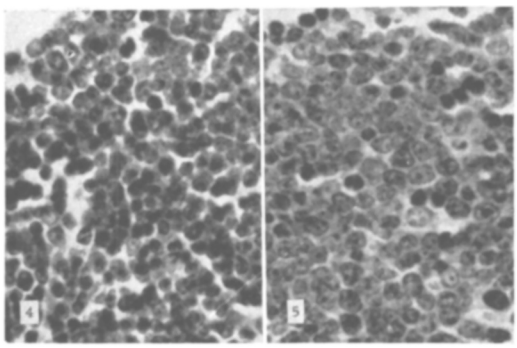
FIG. 2. Uninj., 45-day-old mouse. × 200.
FIG. 3. Virus-inj., 62-day-old mouse. Bio-assay positive. × 200.

voluted thymuses were similar to those of uninjected animals and were presumably due to some stress other than viral infection.

Transplantation assays of thymuses from virus-injected animals were performed for presence of neoplastic cells. Results corroborated the microscopic findings as shown in Table II. These assays failed to disclose the presence of neoplastic cells before 57 days of age, with 3 probable exceptions.

One 35-day-old thymus produced local tumors at graft sites, measuring 0.5 and 1.0 cm in 2 of 4 recipients, 91 and 152 days after grafting. These recipients were 5 and 7 months old at death. One also had thymic lymphoma and generalized leukemia. A third recipient of this thymus developed generalized leukemia at 5 months of age, 90 days after grafting, with questionable thymic enlargement.

One of 4 recipients of a 50-day-old thymus developed generalized leukemia at 4½ months of age, 76 days after grafting, without tumors at graft sites.



From the thymus of AK/Z mice

FIG. 4. Same as Fig. 2. × 550.
FIG. 5. Same as Fig. 3. × 550.

Microscopic picture of the thymus of a virus-injected mouse at 58 days, judged to be not leukemic, is shown in Fig. 6. Bio-assay of thymus was negative for leukemic cells. In contrast, bio-assays of 68- and 73-day-old mice, clearly leukemic morphologically, were positive (Fig. 8,9). When replacement of normal thymus cells by large (leukemic) lymphocytes begins, it appears to proceed rapidly. An intermediate, conceivably "conditioned," phase is illustrated in Fig. 7. This shows the thymus of a virus-injected 65-day-old mouse with increased number of medium sized lymphocytes, which on bio-assay

TABLE II. Transplantation Assays for Neoplastic Cells in Thymuses of Virus-Injected AK/Z Mice.

Mouse assayed			Recipients					
Age (days) at			Age (days) at			Result of assay*		
Inj.	Assay	Thymus micro.	Graft	Death	Days after graft	Grafted L‡	Spont. L§	Neg.
2	35	N	58	148–210	90–152	3/4	1/4	—
2	36	N	62	178–212	116–150	—	4/4	—
2	36	N	62	187–206	125–144	—	4/4	—
2	50	N	57	133–194	76–135	1/4	3/4	—
2	50	N	57	157–171	103–114	—	4/4	—
1	52	N	36	104	68	—	—	2/2
1	52	N	36	104	68	—	—	2/2
1	53	N	32	137	105	—	1/1	—
2	56	N	36	104	68	—	—	2/2
3	57	N	36	92, 104	56, 68	1/2	—	1/2
3	58	N?	34	137	103	—	1/1	—
3	59	N	32	147	115	—	1/1	—
2	60	±?	43	92, 104	49, 61	1/2	—	1/2
2	60	N?	43	104	61	—	—	1/1
3	62	+	34	75	41	1/1	—	—
1	62	+	40	117	77	1/1	—	—
2	65	±?	39	98	59	—	—	2/2
2	65	+	39	98	59	—	—	1/1
1	66	atr	?	?	65	—	—	2/2
2	67	±?	?	?	65	—	—	2/2
2	67	−	45	95	50	1/1	—	—
3	68	+	40	60	20	1/1	—	—
3	73	+	45	67	22	1/1	—	—
2	74	+G†	?	?	23	2/2	—	—
2	74	+G	?	?	21, 34	2/2	—	—

* No. of mice/No. grafted.

† G = generalized leukemia.

‡ Generalized leukemia without thymic lymphoma, with or without tumor at graft site. All but 4 "positive" in this column had a tumor at graft site.

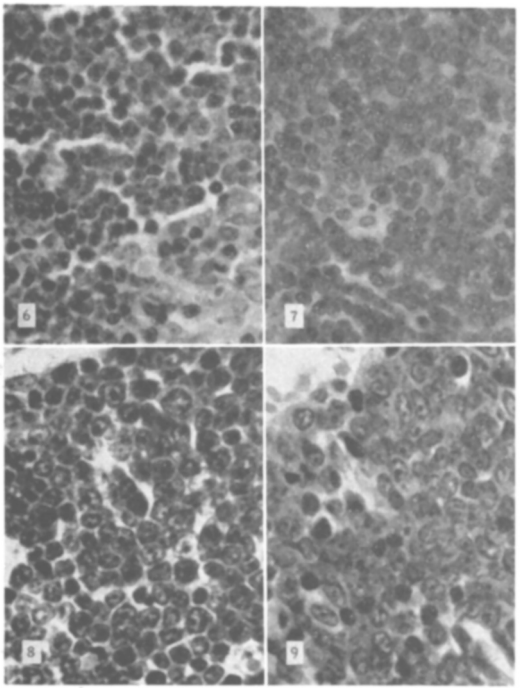
§ Thymic lymphoma with or without generalized leukemia, without tumor at graft site.

proved negative for autonomous leukemic cells. A closer study of this transition period with better cytologic technic is indicated.

All assays considered to be positive yielded local tumors at graft sites, with generalized spread but without thymic involvement, with the 2 following exceptions: One recipient of a 60-day-old thymus, and another of a 74-day-old thymus, developed generalized leukemia without thymic involvement and without local tumors at graft sites at 68 and 34 days, respectively, after grafting.

Worthy of note is development of "spontaneous" thymic lymphoma, in 18 of 22 mice receiving grafts of virus-injected but microscopically normal thymuses. These leukemic mice were 100 to 151 days old at death. Fig. 1 indicates that these leukemias were virus-induced, *i.e.*, the non-leukemic grafted thymuses carried virus. This was not unexpected, since it is known that virus persists in thymectomized mice and that non-leukemic AK mice of both sexes transmit the leukemia "factor" to their offspring(4,5,6).

Summary and conclusions. 1. Incidence of thymic leukemia in AK/Z mice injected as newborns with Gross leukemia virus (agent A) was 90% and the peak incidence of the disease occurred at 3-4 months of age. Incidence of leukemia in the controls was 73% with a peak occurrence at 8-12 months. 2. Thymuses from 52 sacrificed virus-injected AK/Z mice were examined at 13-72 days of age. No gross or microscopic abnormality was noted until about 50 days, when multiple foci of medium to large lymphocytes appeared in the cortex. These foci rapidly became confluent and at about 70 days of age, the thymus was transformed into a lymphomatous tumor. 3. Bioassays of 22, 57-74 day old thymuses from virus-injected mice disclosed autonomous leukemic cells in 10. Correlation of microscopic and bioassay diagnosis of malignant transforma-



From the thymus of AK/Z mice

FIG. 6. Virus-inj. 58-day-old mouse. Bio-assay negative. × 550.

FIG. 7. Virus-inj. 65-day-old mouse. Bio-assay negative. × 550.

FIG. 8. Virus-inj. 68-day-old mouse. Bio-assay positive. × 550.

FIG. 9. Virus-inj. 73-day-old mouse. Bio-assay positive. × 550.

tion was somewhat below 100%. Some thymuses, which were judged to be leukemic "in situ", were not transplantable. 4. Bioassays indicate the presence of virus in non-leukemic thymuses of virus-injected mice. 5. It is concluded that the pathogenesis of virus-induced thymic lymphoma is similar to that of "spontaneous" lymphoma in the high leukemic strain but occurs at an earlier age.

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Steroids and Carbohydrate Metabolism in the Domestic Bird.* (26369)

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Cortisone has frequently been used as a standard reference compound in the study of glucocorticoid activity in the mammal. In such studies, cortisone has invariably been ranked close to hydrocortisone in activity although slightly less potent(1-3). While numerous investigators have reported a high potency for hydrocortisone in the bird(4-7), few have attempted to compare the activity of hydrocortisone and cortisone. In some instances, cortisone has been utilized in studies in the bird(8,9), but no comparison with hydrocortisone was undertaken. Previous results from this laboratory have indicated that cortisone, in contrast with hydrocortisone, possesses little glucocorticoid activity in the bird(7). In addition, Stamler(4) reported a failure to induce hyperglycemia in the chick with cortisone at doses which were effective with hydrocortisone.

In view of an apparent lack of knowledge concerning the relative effectiveness of vari-

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