Effect of Neonatal Thymectomy on Induction of Leukemia by Virus in Rats.* (29980)

A. KUNII, A. CALI AND J. FURTH
Department of Pathology, Columbia University - Francis Delafield Hospital, New York City

Thymectomy of about one month old rats infected with a leukemia virus in the neonatal period reduced the lymphoid leukemia incidence from 97% to 47%, and raised the myeloid leukemia incidence from 0% to 12% (1). It was postulated that during the first 4 to 5 weeks of life, leukemic transformation may have occurred and that some of the lymphoid leukemias in the virus-infected thymectomized rats were derived from the cells which had been transformed in the thymus prior to the thymectomy and metastasized in the other organs. In order to verify this supposition, rats were thymectomized 1 to 3 days after birth and infected with the leukemia virus on the same or next day. In these experiments lymphoid leukemia incidence was reduced from about 85% to about 15%, as will now be described.

Materials and methods. These were essentially the same as in the earlier study(1). As before, pregnant Sprague-Dawley rats were purchased from the Camm Research Institute. The leukemia virus used here was a rat-adapted Gross passage virus(2). A new batch of leukemic filtrate containing the virus was prepared from pooled thymuses of rats with thymic lymphoma induced previously(1) according to our standard procedures(3), frozen in ampules and thawed as needed. Approximately two-thirds of each litter of newborn rats were thymectomized 1 to 3 days after birth, the rest being kept unoperated as controls. The same or next day all rats were injected intraperitoneally with 0.05 ml of the leukemic filtrate thawed shortly before injection. The thymectomy was done as described by Dischler and Rudali(4).

The infectivity of a batch of the leukemic filtrate used in these experiments was tested in groups of 15-20 newborn rats with results as follows: 0.05 ml of the undiluted filtrate induced leukemia in 93.3%, 10^{-1} dilution in 78.6%, 10^{-2} dilution in 42.1% and 10^{-3} dilution in 33.3%. Tissue culture medium 199 was used as a diluent. The surviving rats in this experiment were sacrificed 7 months after virus-infection. Higher dilutions were not tested.

Nursing mothers received bread and milk daily. The rats were weaned at 4 to 5 weeks of age and the males and females were separated. Rats with intercurrent infections were given Terramycin (animal formula, Pfizer) in the drinking water and/or injections of Bicillin (Wyeth).

Some of the thymectomized virus-infected rats were treated various ways about 2 weeks after virus-infection in attempts to restore sensitivity to leukemia. These preliminary trials made on a small scale were uniformly unsuccessful. In most of these trials Millipore® filter (pore size 0.45 μ) diffusion chambers, either empty or containing thymus from newborn rats were used, prepared according to the method of Levey *et al*(5) and inserted intraperitoneally. A small number of the thymectomized virus-infected rats received either an intraperitoneal graft of thymus from a newborn rat or an intraperitoneal injection of thymic lymphocytes of one-week-old rats. The experiments were terminated 28 to 29 weeks after virus-infection, when all surviving rats were sacrificed, carefully autopsied and examined microscopically for leukemia.

Results. Two sets of experiments were performed in succession (Tables I and II). The number of rats that died before detection of the first leukemia were discounted from the total. At the end of the second week of life 18% of the unoperated virus-infected rats were dead in Experiment I and 11% in Experiment II. By the end of the 5th week the cumulative mortality was 30% and 20% respectively. There was no further increase in mortality until the first leukemic death occurred. In contrast, the cumulative mortality of the thymectomized virus-infected rats was 45% in Exp. I and 22% in Exp. II at the

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TABLE I. Incidence of Leukemia in Thymectomized and Unoperated Virus-Infected Rats (Experiment I).

		No. of rats	Lymphoma No.	Lymphoma %	Latency in days Mean (Range)
I	Unoperated	35	26	74.3	89 (60-185)
II	Thymectomized	38	5	13.2	126 (92-160)
III	Thymectomized and given:				
	a) Millipore chamber containing thymus	23	2	8.7	100 (90-110)
	b) Empty Millipore chamber	12	1 (2)*	8.3 (16.6)*	120 (150)*
	a) and b) combined	35	3 (4)*	8.6 (11.4)*	

* Figures in parentheses include one case of myeloid leukemia.

TABLE II. Incidence of Leukemia in Thymectomized and Unoperated Virus-Infected Rats (Experiment II).

		No. of rats	Lymphoma No.	Lymphoma %	Latency in days Mean (Range)
I	Unoperated	43	40	93.0	83 (50-200)
II	Thymectomized	21	4	19.0	130 (110-160)
III	Thymectomized and given:				
	a) isologous thymus graft, i.p.	4	1	25.0	200
	b) isologous thymocytes, i.p.	8	1	12.5	200
	a) and b) combined	12	2	16.7	

end of the second week of life, increasing gradually to 65% and 40% respectively by the 7th and 8th week of life. The various additional treatments given at 2 weeks of age did not change the mortality rate. A common infection observed during the pre-leukemic period was massive acute lobar pneumonia with fibrinous pleurisy seen in both operated and unoperated rats, occurring at higher frequency in Exp. I than in Exp. II. Mediastinal, pulmonary and subcutaneous abscesses at the site of skin incision were other complications encountered among the operated rats.

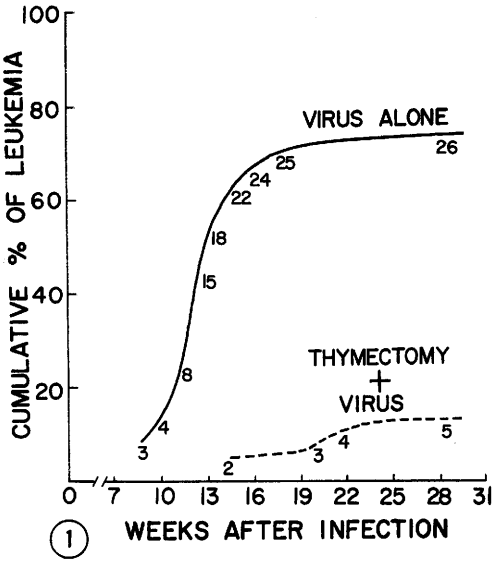
Thymectomy was incomplete in 2 rats of Exp. I and 5 of Exp. II as indicated by development of characteristic thymic lymphomas. These were counted as unoperated rats.

Neonatal thymectomy reduced the leukemia incidence from 74.3% to 13.2%. Millipore chamber with or without thymus lowered still further this incidence (Table I); however, only few presumably "living" cells were seen in section of thymuses in these chambers. All but one leukemia of this series was lymphoid. A myeloid chloroleukemia occurred in a rat that was thymectomized, virus-infected, and given an empty Millipore chamber. The lower than usual incidence of leukemia in unoperated virus-infected rats in Exp. I (74.3% *vs* 93.0% in Exp. II and 93.3% in the titration test) can be explained by the relatively high frequency of intercurrent infections, mostly pneumonia. It has been shown earlier that pneumonia and other diseases associated with involution of the thymus lower the leukemia incidence(6).

Experiment II (Table II) confirmed the results of Exp. I. The incidence of leukemia was higher than in Exp. I in both thymectomized (19% *vs* 13.2%) and unoperated virus-infected rats (93.0% *vs* 74.3%). The number of rats with thymus graft is small but the findings agree with studies reported by others in mice (cf. 6). A similar study is now being carried out on a larger scale in attempts to restore sensitivity of thymectomized rats to leukemia.

Fig. 1 and 2 show the time table of leukemia development in Exp. I and II.

Discussion. The experiments described here support the idea that leukemic transformation of cells begins soon after virus-infection and that many leukemias occurring in rats thymectomized several weeks after virus-infection may have originated in neoplastic cells which escaped from the thymus before



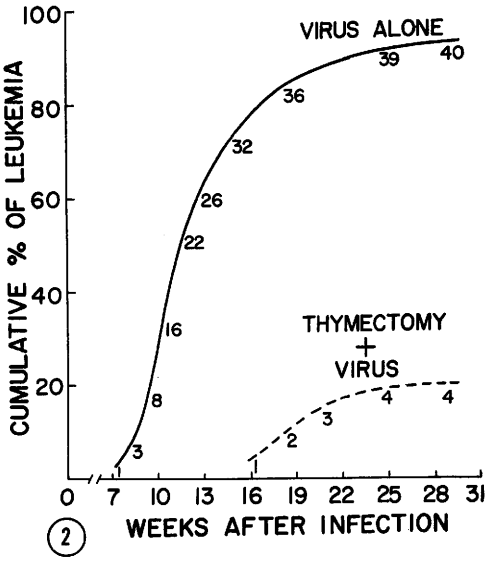


FIG. 1. Cumulative development of leukemia in unoperated and thymectomized virus-infected rats in Experiment I. The number along the curve indicates number of rats with leukemia.

FIG. 2. Cumulative development of leukemia in unoperated and thymectomized virus-infected rats in Experiment II. The number along the curve indicates cumulative number of rats with leukemia.

thymectomy. In the spontaneous leukemias of the AK mice, from which this virus originates, thymectomy performed at about 6 weeks of age causes a decrease in leukemia incidence(6) as profound as achieved only by neonatal thymectomy in rats and mice infected with the passage virus. It follows from this and experiments of others(6) that the time and frequency of leukemic transformation depends on the virulence and/or concentration of the virus. (There are no studies on record to distinguish clearly between virulence and concentration-effect of leukemia virus.) Electron microscopic study suggests that the high virulence of this passage virus is associated with abundance of virus(2).

The present experiments also support the idea that this virus can induce leukemias in hemopoietic cells other than thymic lymphocytes. (The thymectomy was performed before virus-infection.) The possibility that in thymectomized rats with non-thymic lymphomas there was ectopic thymic tissue somewhere in the body(7) cannot be excluded although careful autopsies disclosed none. Earlier experiments indicate that the virus persists in thymectomized hosts, and that their offspring are just as liable to leukemias as those of non-thymectomized parents(8). Thus the thymic factor, yet to be identified, is merely a promoter. Conclusive evidence is still lacking that this thymic non-cellular factor is a hormone, as supposed by some. Attempts to demonstrate a leukemogenic capacity in cell-free thymic extracts(9,10) have thus far been inconclusive as was an attempt to induce leukemia by means of parabiosis of a thymectomized with a non-thymectomized AK mouse(11). In the present experiment thymuses enclosed in the Millipore chambers failed to restore sensitivity to leukemia. Since the thymus grafts in these chambers became almost entirely necrotic, this experiment does not invalidate the work of others who noted restoration of immunologic capacity by Millipore filter chamber in which the thymus survived(7).

The present experiments also suggest that the stress factor(6), which is known to operate by ACTH mediated cortisone discharge, causing atrophy of the thymus, also functions in thymectomized animals, *i.e.*, it causes a reduction of non-thymic lymphomas. Such an effect is to be expected since glucocorticoids are generally lymphocytocidal although the thymus is their most sensitive target.

However, the available data are merely suggestive and further experiments are needed to prove this supposition. If this hypothesis is correct, adrenalectomy (with substitution for lack of mineral corticoids) should increase the incidence of non-thymic lymphomas in neonatally thymectomized hosts.

The incidence of myeloid leukemias was markedly increased by thymectomy in our earlier experiment(1) and in those by Gross (12). In the present experiment only one case of myeloid leukemia was found in 106 thymectomized virus-infected rats. It occurred in a thymectomized rat with an implanted empty Millipore chamber. The lower incidence of myeloid leukemia in the present experiment (less than 1%) compared with 12% in our earlier experiment(1) is puzzling. We expected a higher incidence because more rats escaped early deaths from non-thymic lymphomas (cf. 6). Failure of thymectomized rats to develop myeloid leukemia in the present experiments may be explained by low concentration of the virus. The thymus is a favored organ for replication of the virus(2). If so, neonatal thymectomy prior to virus-infection as done in the present experiments, eliminates this effect of thymus and neonatally thymectomized rats might be expected to have less virus than rats infected with virus first and thymectomized one month later.

Summary. 1. Neonatal thymectomy reduced the induction rate of leukemia by virus from 74.3% to 13.2% in one experiment and from 93.0% to 19.0% in another. 2. This indicates that many non-thymic lymphomas occurring in rats thymectomized one month after virus-infection(1) originated in cells which have been transformed in the thymus and seeded in other lymphoid organs prior to thymectomy. 3. The data also suggest a) that non-thymic lymphomas may be induced by the virus and b) that stressful agents which cause corticoid discharge might lower not only the incidence of the thymic, but also the non-thymic lymphomas.

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Preparation and Properties of a Small-Particle Aerosol of Coxsackie A_{21}. (29981)

ROBERT B. COUCH, PETER J. GERONE, THOMAS R. CATE, WILLIAM R. GRIFFITH, DAVID W. ALLING AND VERNON KNIGHT

U. S. Department of HEW, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboraory of Clinical Investigations, Bethesda, Md., and U. S. Army Biological Laboratories, Ft. Detrick, Md.

A variety of inoculation methods have been used in previous volunteer studies with respiratory viruses(1). While these studies often resulted in virus infection and illness, they were limited by the uncertainty regarding the location and dose of virus delivered to the