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Studies on the Mechanism of Action of Thymic Leukemogenic Virus.* (31172)

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At the present time little is known of the mechanisms by which leukemia develops in man. By the time clinical signs first appear, the disease is relatively far advanced. Fortunately, the laboratory mouse offers a convenient model in which to study leukemogenesis(1). Potent viral agents are available which induce leukemia in high incidence after a short latency(2). When these viruses are inoculated intraperitoneally into susceptible mice, a thymic lymphoid leukemia develops. When the same virus is given to thymectomized mice, a myeloid leukemia occurs(3,4).

The dynamics of the evolution of the thymic lymphoma are currently under study and several steps are already known. Within a day after virus inoculation, the titer of infectious (input) virus in tissues is significantly reduced. This period of "eclipse" is followed by a generalized viremia, at which time virus is present in the blood and may be found in all tissues examined. This viremia occurs many weeks before the earliest histologic appearance of tumor. Under these conditions it would appear that each of the 2 thymuses is equally exposed to the virus. It was therefore surprising to observe that the leukemogenic response was almost always unilateral, occurring in only one of the 2 thymuses(5,6).

In the development of thymic lymphatic leukemia, two types of mechanisms may be considered. The leukemogenic effect of the virus may be *direct,* acting on the cells of the individual thymus where the tumor first appears, or the virus may act *via* an *indirect* systemic leukemogenic action, perhaps mediated through immunologic mechanisms. The unilateral development of tumor in the face of a generalized viremia suggested the possibility of an indirect leukemogenic action(6). Recent studies by Lieberman, Haran-Ghera, and Kaplan suggested that a direct viral effect was operative(7). If the virus acts *directly* on the thymic cells, then inoculation of virus into one of the thymuses should produce the tumor in that thymus and not in its mate. In the present quantitative study, we have confirmed and extended the report of Lieberman *et al,* and present additional data which support the hypothesis that the leukemogenic virus acts directly *within* the inoculated thymus.

Materials and methods. A murine leukemia virus (Rich) was employed as previously described(8). A 100 μl syringe was fitted with a 30 gauge needle which had been reground

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TABLE I. Distribution of Lymphoma Following Unilateral Intrathymic Virus Inoculation.

	Group used for analysis, see text ↘			
	Tumor-free	Unilateral thymic lymphoma	Bilateral thymic lymphoma	Disseminated lymphoma
Inoculated in right thymus	17	18*	5	7
Inoculated in left thymus	10	12†	9	19
Totals	27	30	13	26

* In 3 cases early splenic metastases present. † In 4 cases early splenic metastases present.

to provide a blunt bevel. Three- to six-day-old Ha/ICR Swiss mice random-bred in our laboratory were lightly anesthetized with intraperitoneal Nembutal. The mice were restrained on a board in the ventral position with plastic tape, and the sternum opened from above with a fine scissors. By slanting the sternal cut slightly to the left or right, the left thymus or right thymus appeared directly in the incision. To minimize leakage, inoculations were made in the longitudinal axis of the thymuses. From 2 to 5 μl of undiluted virus stock were inoculated into the right or left thymus, and the corresponding ear cut for identification. An inoculation was considered satisfactory when the inoculated thymus swelled slightly during inoculation and little leakage occurred. The skin edges were closed with 2 fine black silk sutures. To avoid premature removal of the sutures by the mother, the litter was kept warm under a lamp for 6 hours, after which the animals were returned to the cage. Postoperative mortality was slight.

The experiment was terminated between 51-73 days after inoculation, at which time previous studies had shown that the maximum number of animals would show the *early* lymphoma changes(4,6). If animals were sacrificed earlier, definite lymphoma would be rare, whereas if sacrifice was delayed, only generalized lymphoma would be found. The left thymus, right thymus, and spleen were weighed and processed for histology. The criteria employed for histologic diagnosis of lymphoma have been published (6). In brief, a thymus was considered neoplastic when all the normal thymic lymphocytes were absent, and the entire thymic mass was completely replaced by large, primitive lymphoblasts. The tumor, once initiated in one thymus, rapidly spread to the opposite thymus, the spleen, and other organs. The rapid spread of the tumor, once initiated, and the variability in latency, introduce technical difficulties in the collection of data. For this reason, this study was specifically designed to compare only the incidence of tumor *arising* in the *inoculated* thymus with that *arising* in the *uninoculated* thymus. Only those animals in which the tumor was confined to one thymus could therefore be used. Cases of bilateral thymic tumor were excluded because it could not be determined in which thymus the tumor arose, or whether it arose bilaterally.

Results and discussion. For this study only overt, well-established lymphoma was considered positive. Cases of lymphoma-*in situ* (6) were excluded. Animals were placed in one of 4 groups, depending on the stage of lymphoma found by histology, as shown in Table I. Of the 96 animals studied, 69 had lymphoma. Of these 69, 13 had tumor in both thymuses and therefore were not suitable for further analysis, as discussed above. In 7 cases of unilateral thymic lymphoma, early dissemination of tumor had already occurred to the spleen, but the thymus tumor had not yet invaded the opposite thymus, and the identification of the thymus of origin could thus be made. These 7 animals, even though early dissemination had occurred, were therefore included with the Unilateral Thymic Lymphoma group in Table I. In 26 cases, far-advanced dissemination of tumor to all organs had occurred, which precluded their use for this study. Thirty cases of lymphoma were obtained in which the lymphoma was limited to one thymus, and these thymuses could therefore be assumed to be the site of origin. These 30 animals were further

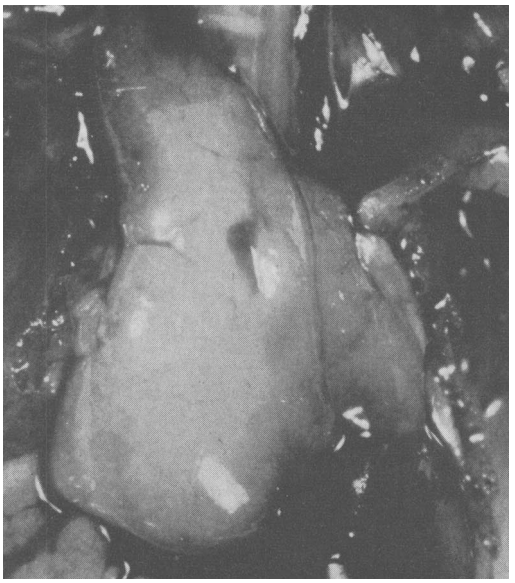

FIG. 1. Gross appearance of the 2 thymuses after virus inoculation in right thymus. Right thymus is markedly enlarged and weighs 84 mg. The uninoculated left thymus (reader's right) is normal in size and weight for the strain. See Fig. 2 for histology.

analyzed in Table II to determine the influence of the site of virus inoculation (right or left thymus) on the site of tumor evolution. As may be seen, there is a marked preponderance of tumors occurring in the inoculated

TABLE II. Relation of Unilateral Tumors to Thymus of Inoculation.

	Tumor in right thymus	Tumor in left thymus
Inoculated in right thymus	17	1
Inoculated in left thymus	2	10
Total tumor in inoculated thymus		27
Total tumor in uninoculated thymus		3

thymus. Of the 30 cases, the inoculated thymus developed the tumor in 27 instances (90%), whereas the uninoculated thymus developed the tumor in only 3 cases.

The gross appearance of a specimen in which the inoculated thymus had developed a lymphoma is illustrated in Fig. 1. The inoculated right thymus weighed 84 mg, and was completely replaced by lymphoma. The uninoculated, normal left thymus weighed 20 mg, which is normal for the age and sex of these mice. The histology of the tumor-filled, inoculated right thymus may be contrasted with the appearance of the normal lymphocytes in the uninoculated left thymus, as illustrated in Fig. 2.

In this study a considerable number of animals at the time of sacrifice had tumor

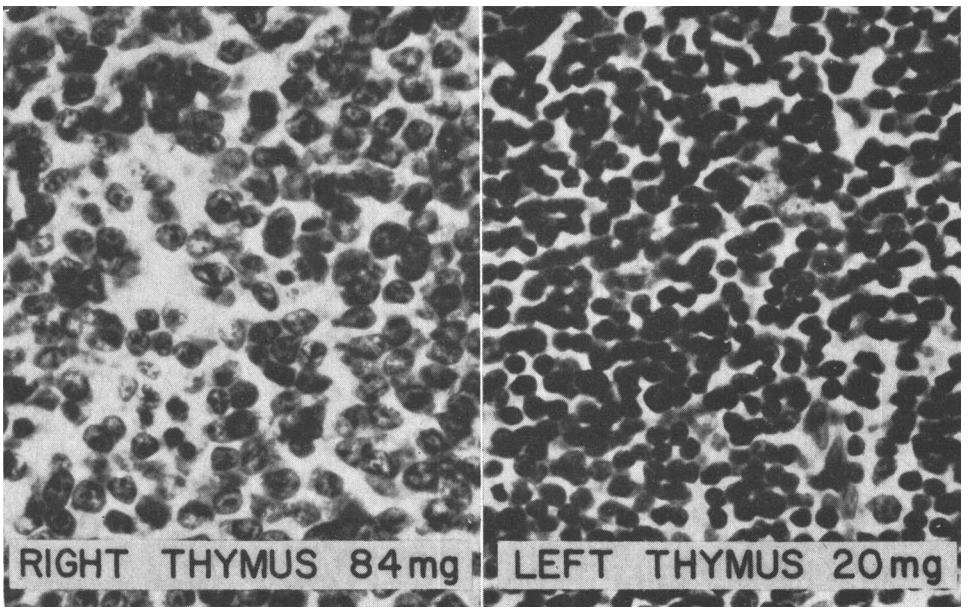

FIG. 2. Right thymus is filled with large, primitive tumor lymphoblasts. Left thymus contains only normal lymphocytes.

in both thymuses (13 of 69). In earlier studies bilateral thymic tumor evolution was rare following *intraperitoneal* virus inoculation(6). If these cases of bilateral thymic tumor in this study represent bilateral *evolution,* rather than unilateral evolution and subsequent direct spread, then a possible explanation may be leakage of virus during the intrathymic inoculation.

While this experiment was not designed to determine the latent period between inoculation and tumor development, it was noted that generalized lymphomas were already present in many cases by 55 days of age. This finding suggests that the latency of lymphoma following intrathymic inoculation is significantly shorter than that observed following intraperitoneal inoculation.

The consistent occurrence of tumors in that thymus which had been inoculated with the virus supports the view that the mechanism of viral leukemogenesis is a direct one.

If the mechanism is direct, it remains to explain the consistent *unilateral* evolution of thymic lymphoma following intraperitoneal inoculation where presumably both thymuses are exposed to virus. Two possibilities may be considered: the virus was able to enter only one thymus, or virus could enter both thymuses but one thymus was physiologically more responsive than the other. To evaluate the first possibility we are at present determining the time sequence for appearance of virus in each of the 2 thymuses. Preliminary assay results have indicated that following intraperitoneal inoculation, the amount of infectious virus in each of the 2 thymuses differs considerably, and preliminary electron microscopic studies support this observation (9).

Summary. Murine Leukemia virus (Rich) was inoculated into only one of the 2 thymuses of infant mice which were sacrificed 2 months later. The animals were analyzed to compare the incidence of lymphoma evolving in the inoculated thymus with the incidence of lymphoma evolving in the uninoculated thymus. It was found that of 30 animals suitable for analysis, in 90% of cases the tumor arose in the inoculated thymus, supporting the view that the mechanism of viral leukemogenesis is *direct.*

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Pancreatic Lipase Activity in Deuterium Oxide.* (31173)

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In most of the studies on enzymatic activity in D_2O, soluble substrates have been employed. However, there are a large number of enzymes that act on insoluble substrates, for which the conventional Michaelis-Menten kinetic analysis is inappropriate(1). One of these is pancreatic lipase (glycerol ester hydrolase, EC 3.1.1.3), shown by Sarda and Desnuelle(2) to act only on emulsified esters; although crude preparations could hydrolyze

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