Studies on Restoration of Sensitivity of Thymectomized Rats to Viral Leukemia

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Summary

Thymectomy at 3–4 days of age prevented the induction of leukemia by virus in WF rats; grafts of neonatal thymus corrected this deficiency. Multiple thymus grafts were much more effective than single ones. Similarly, thymectomy in rats caused a lasting reduction of lymphocyte levels and the capacity to produce hemagglutinins; these deficiencies were also corrected by thymus grafts. In restoring susceptibility to leukemia and the lymphocyte levels, 5 thymuses were more effective than a single thymus, and 10 thymuses were more effective than 5 thymuses. In restoring immunologic capacity, 5 and 10 thymuses were more effective than a single thymus.

Transplantation assays of the induced lymphomas for genetic and histocompatibility factors suggested that the induced lymphoma cells were of 3 types: (a) those which possessed the transplantation pattern of the donor cells; (b) those which had the transplantation pattern of the host; and (c) those which were immunologically altered. This alteration is indicated by lack of transplantability in adult rats with transplantability in young rats and/or by regression of tumors in adult hosts.

Electron micrographs revealed the presence of replicating virus in all 3 lymphomas arising in thymus grafts that were examined. The mechanism by which the thymus and the virus bring about neoplastic transformation of normal lymphoid cells remains conjectural.

Introduction

Experiments in mice indicated that thymectomy prevents the spontaneous development (5, 27, 35) and induction (5, 10, 17, 28, 33, 41) of leukemia in most mice, that grafts of thymus can correct this deficiency (11, 19, 27, 28, 32, 40), and that leukemic cells in thymectomized mice grafted with thymus often originate in the host's lymphoid cells (4, 18, 29, 42, 45). The extensive literature on the role of thymus in leukemogenesis has been fully reviewed elsewhere (6, 12, 44).

Thymectomy at birth creates immunologic incompetence, which can be similarly restored by thymus grafts (2, 7, 43, 46). Since thymic tissue introduced in Millipore diffusion chambers restored immunologic competence (1, 30, 31, 50, 51), it was suggested that the thymus secretes a hormone-like substance, which brings about this effect.

Experiments with rats are being undertaken by us to demonstrate the existence of a substance capable of restoring sensitivity to thymectomized hosts to induction of leukemia by virus and to learn about the relation of such a substance to that which can correct the immunologic deficiency in neonatally thymectomized rats.

The 1st series of experiments utilizing Millipore diffusion chambers were unsuccessful because of the poor survival of the thymus in these chambers (23). Therefore, preliminary experiments were performed to verify that in rats, as in mice, leukemias induced by thymus grafts in thymectomized hosts often originate in cells of the hosts. Furthermore, since the thymus grafts were reported not to be under homeostatic control (37, 38), the possible enhancing effect of multiple grafts on leukemia induction was investigated. Results of these experiments are reported in this communication with data on the immunologic capacity and levels of peripheral lymphocytes of thymectomized rats with and without thymus grafts.

Materials and Methods

Animals. The rats used were of the highly inbred Wistar/Fu (W/Fu) and Fisher/Fu (F/Fu) strains and 1st generation hybrids of W/Fu female x F/Fu male (WF). The W/Fu and F/Fu rats were purchased, 4 and all newborn rats were reared in our laboratory.

Virus. A rat-adapted passage A virus of Gross (49) was used. The preparation of the leukemic filtrate containing the virus and its storage were described earlier (23, 33). The filtrate used throughout the present experiments was from the same lot as that used in an earlier study (23).

Thymectomy, Thymus Implantation, and Virus Infection. In Experiment 1 (Chart 1), on the restoration of leukemia induction in thymectomized and virus-infected rats by a single thymus graft, newborn WF rats were neonatally (within 3–4 days of age) thymectomized by the same method as used in an earlier study (23). At the same time, a neonatal thymus of parental type was implanted s.c. in the right flank. The sex of the thymus donor was the same as that of the recipient. On the same or on the

4 From A. R. Schmidt Co., Inc., Madison, Wis.
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**Chart 1.** Cumulative leukemia incidence in nonthymectomized and thymectomized WF rats with and without thymus grafts (Experiment 1). The rats were thymectomized at 3–4 days of age and grafted with neonatal thymus of parental type, followed by virus infection on the same or the next day.

Following day these rats were given i.p. injections with 0.05 ml of virus filtrate. Simultaneous controls set up within the same litter included virus-infected (“positive control”) and thymectomized and virus-infected (“negative control”) animals.

In Experiment 2 (Chart 2), in which the effects of multiple and single thymus grafts on leukemia induction were compared, the newborn WF rats were thymectomized at the age of 3–4 days with a wire loop snare (53) under light hypothermia. The wire loop snare (Fig. 1) was made of a stylet for 20–24 gauge needles held by an 18–22 gauge needle. The size of the loop varied according to the size of the thymus. Thymectomy with the wire loop snare has 2 advantages over conventional surgical thymectomy: namely, less bleeding and shorter operation time. These enable the use of a lighter degree of hypothermia, which greatly reduces postoperative mortality and the number of incomplete thymectomies.

The siblings that were not operated upon (“positive controls”) were also subjected to hypothermia. After recovery from hypothermia, all newborns were given i.p. injections of 0.05 ml of virus filtrate. About 2–3 weeks later, 1, 5, or 10 neonatal thymuses of either W/Fu or F/Fu rats were grafted subcutaneously on thymectomized, virus-infected rats. The sex of the donor was matched with that of the recipient.

In a comparative study of the effects of single and multiple thymus grafts on peripheral lymphocyte levels and immunologic capacity of neonatally thymectomized rats, noninfected F/Fu rats were similarly thymectomized at 3–4 days of age. The isologous neonatal thymus grafts, matched for sex, were made at 3 weeks of age. All rats were weaned and separated according to sex at 4 weeks of age.

**FOLLOW-UP OF VIRUS-INFECTED WF RATS.** Every other week, or weekly, the rats were inspected for leukemia development and palpated for grafts, and blood smears were made. The experiments were terminated by sacrificing the survivals 200 days after virus infection.

**TRANSPLANTATION ASSAY.** In order to determine whether the
TABLE 1

TRANSPLANTATION PATTERN OF LYMPHOMAS ARISING AT SITES OF THYMUS GRAFTS

<table>
<thead>
<tr>
<th>No. of Lymphoma</th>
<th>Recipients</th>
<th>Type</th>
<th>Observation Period (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-3 mos old</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>WF</td>
<td>W/Fu</td>
<td>F/Fu</td>
</tr>
<tr>
<td>1</td>
<td>5(1)/0a</td>
<td>0/4</td>
<td>0/4</td>
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<tr>
<td>2</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
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<td>3</td>
<td>0/5</td>
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<td>0/5</td>
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<td>4a,b</td>
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<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td>4b</td>
<td>4/4</td>
<td>4(2)/4</td>
<td>5/5</td>
</tr>
<tr>
<td>5</td>
<td>0/3</td>
<td>4(2)/6</td>
<td>9/19</td>
</tr>
<tr>
<td>6</td>
<td>0/5</td>
<td>0/5</td>
<td>0/6</td>
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</tr>
<tr>
<td>8</td>
<td>3/12</td>
<td>0/4</td>
<td>8/8</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td>1/10*</td>
</tr>
<tr>
<td>10</td>
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<tr>
<td>15</td>
<td>2(2)/5</td>
<td>4(3)/6</td>
<td>2(1)/6</td>
</tr>
<tr>
<td>16</td>
<td>0/4</td>
<td>0/5</td>
<td>3 (alive)4</td>
</tr>
<tr>
<td>17*</td>
<td>9/9</td>
<td>0/4</td>
<td>3 (alive)</td>
</tr>
</tbody>
</table>

* Number of takes over number of rats grafted. The figures in parentheses indicate the numbers of rats in which the tumors regressed after reaching an approximate size of 15 mm across. The regressed tumors are counted among the takes.

** Lymphoma 4a, obtained by surgical excision 107 days after thymus grafting, weighed 1.8 gm. Lymphoma 4b, obtained 68 days later, weighed about 5 gm.

† This rat with lymphoma was the only one grafted at 16 days of age. It died with generalized lymphoma 8 days later. Six young F/Fu rats that died at 4-6 days after grafting of intercurrent disease are not listed.

* The rats listed as alive will probably be free from grafted lymphoma since the latency of tumor takes in adults varied from 2-6 weeks and in very young rats from 1-4 weeks.

In this case, there was no tumor at the size of the graft; spleen and lymph node cells were used for the transplantation assay.

Leukemias originated in lymphoid cells of the donor or of the host, 16 lymphomas arising in the thymus grafts and 1 generalized lymphoma without involvement of the grafted thymus were transplanted in adult (3-8-week-old) WF and W/Fu or F/Fu rats (whichever was the thymus donor). In 6 instances, young rats (3-14 days old; in 1 instance, 16 days old) were used as hosts to detect immunologic modification in leukemic cells, as suggested by the work of Loachim *et al.* (16) (Table 1).

HEMATOLOGIC AND IMMUNOLOGIC TESTS. Peripheral lymphocyte evels of F/Fu rats were determined on blood from the tail before and at various intervals after thymus grafts. Before termination of the experiment, when the rats were 160-170 days old, several rats of each experimental group were given a single i.v. injection of 150 × 10⁶ washed sheep erythrocytes. One week later, they were exsanguinated and their hemagglutinin titers determined. These 2 tests were also carried out on WF rats surviving 200 days after virus infection.

FIG. 2. Microscopic appearance of a characteristic lymphoma at the site of the thymus graft. This rat was thymectomized and infected at 3 days of age, received subcutaneous grafts of 10 1- to 3-day-old thymuses, and died at 74 days of age. The lymphoma arising at the thymus graft measured 4 X 3 X 2 cm. X 650.

FIGS. 3-5 are from a rat that was killed with lymphoid leukemia with hepatomegaly and splenomegaly 146 days after infection. The lymphomas arising at the thymus graft measured 4 X 3 X 2 cm. X 650.

FIG. 6-8 are from a rat that died 104 days after infection. The 4 grafted thymuses were matted together. Some were normal; others were diffusely infiltrated (Fig. 6, X 20; Figs. 7-8, X 100).
Gomori's buffer, counterfixed with osmium tetroxide, and embedded in Epon (Resin 812, Shell Chemical Co.).

Results

RESTORATION OF LEUKEMIA SENSITIVITY OF THYMECTOMIZED RATS BY SINGLE AND MULTIPLE THYMIC GRAFTS. In Experiment 1 (Chart 1), the incidence of leukemia was 0 in 17 thymectomized rats; in the controls it was 62.9%. A single neonatal thymus graft raised the leukemia incidence in 35 rats to 42.9%. All leukemias were lymphoid. Every litter in the control series yielded a high percentage of leukemias, except for a litter of 11 rats in which only 1 lymphoma was discovered at the termination of the experiment. This explains the lower total percentage of leukemia in the control group than in earlier experiments (13, 24).

As already stated, the hosts were W/Fu × F/Fu hybrids. The thymus donors were either W/Fu or F/Fu rats. Both were about equally effective in restoring sensitivity to leukemia. Seven of the 18 hosts that received W/Fu grafts and 8 of the 17 that received F/Fu grafts developed lymphoma.

The results of Experiment 2 are shown in Chart 2. The incidence of leukemia in the controls was 87.5%; in thymectomized rats given 10 thymus grafts, 58.8%; in rats given 5 thymus grafts, 41.7%; in rats given 1 thymus graft, 31.3%; and in rats without thymus grafts, 8.3%. The total number of rats in the last (control) group was small, but they were brothers or sisters of the rats allocated systematically to the various groups, and the data merely confirm those of several identical earlier experiments (23, 24).

In general, the mean latency period was inversely related to the number of thymuses grafted, being 96 days in the rats not operated upon and 116, 134, 150, and 200 days in the rats receiving 10, 5, 1, and no thymus grafts, respectively.

MORPHOLOGY. All but 1 of 59 leukemias in Chart 1 were thymic lymphomas, occurring in either the thymus or the thymus graft. The largest lymphoma arising at the graft site weighed 63 gm. It is noteworthy that in this rat only the regional lymph nodes showed gross evidence of lymphoma. In 1 thymectomized rat with generalized lymphoma the graft could not be identified. In most cases there was generalized lymph node enlargement, splenomegaly, and diffuse infiltration of the liver. Solitary lymphomatous tumors were rare. The bone marrow was usually infiltrated. In about 3 of the cases in which blood smears were made, lymphoblasts (presumably leukemic) were present in the blood in variable numbers.

All thymus grafts of all rats sacrificed at about 200 days of age were studied microscopically. Neoplastic cells, often examined in imprints, resembled lymphoblasts of the circulating blood.

The characteristic microscopic appearance of lymphomas at the graft site is shown in Fig. 2. When the rats died of lymphoma, the thymus grafts were almost invariably lymphomatous, even though the genetic assays suggested that the lymphoma cells were often of the host cell type, as will be described. An exceptional case is described and illustrated below (Figs. 3–5). In many cases some of the grafted thymuses became lymphomatous, whereas adjacent thymuses retained the normal appearance (Figs. 6–8). Thus, this “accidental” involution can involve a grafted thymus, but does not uniformly affect all thymuses grafted on the same host.

In 1 case, in which the thymectomy was incomplete and a lymphoma arose in the thymic remnant, some grafted thymuses were normal appearing and others were moderately involuted (Figs. 9–11). In 3 cases, a lymphomatous tumor massively infiltrated the thyroid gland and adjacent structures, but it could not be established with certainty that the lymphoma originated in the thyroid (Fig. 12). We considered the possibility that this tumor might have originated in an ectopic thymus in the thyroid, the existence of which in mice has been reported (26). In a moderate number of rats studied in the course of other experiments, ectopic thymuses were not seen in the thyroid. In general, the grafted thymuses retained the normal architecture (Fig. 13), but some were involuted.

Electron micrographs have shown the characteristic virus-like particles in all 3 thymuses that were studied. They were most frequently seen in areas of degeneration, in free intercellular spaces (Figs. 14, 15), and in membrane-lined vesicles (Figs. 16, 17) and budding on villous processes (Figs. 20, 21). They were also seen budding on the plasma membrane of lymphoblasts (Figs. 18, 19, 22, 23), but they were not seen inside lymphocytes or in well-preserved stromal cells. The virus particles in these lymphomas were not as abundant as in the primary thymic lymphomas induced by this virus in our earlier studies (49).

TRANSPLANTATION ASSAY FOR IMMUNOGENIC CHARACTER OF INDUCED LYMPHOMAS. The results of transplantation assays of 16 lymphomas arising at the site of the grafted thymus and of 1 generalized lymphoma are surveyed in Table 1. These are in general agreement with those reported in mice, some of the lymphomas being of the donor, others of the host, type. Recent findings on immunologic modification in viral leukemias led us to use immunologically deficient newborn rats as well. Some of these accepted the grafts, but all the adults rejected them. These findings, as well as the tumor regressions, are interpreted as strongly suggestive of some immunologic modifications of the lymphoma cells, as will be discussed. A more precise analysis of the immunogenetic character of the lymphomas was beyond the scope of the present study.
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**Chart 3.** Mean blood levels of small lymphocytes in nonthymectomized and thymectomized F/Fu rats with 10, 5, 1, or no thymus grafts. The numbers in parentheses indicate the number of rats in each group.

**Lymphopenia in Thymectomized Rats and Its Correction by Thymus Grafts.** The effect of single and multiple thymus grafts on the lymphocyte level of noninfected F/Fu rats thymectomized at 3–4 days of age was studied to obtain some background information on similarly treated virus-infected rats. It is clearly indicated by Chart 3 that the lymphocyte level is depressed by thymectomy and elevated by thymus grafts in direct relation to the number of thymuses grafted. This experiment was terminated at 160–170 days, covering fully the period of thymic lymphoma induction by virus.

The lymphocyte levels in the leukemia restoration series were determined 200 days after thymectomy, when the experiment was terminated (Chart 4). The results are in essential agreement with those given in Chart 3. Thus, there is generally a parallelism between restoration of lymphocyte levels (Charts 3, 4) and incidence of lymphoma (Chart 2) in thymectomized rats grafted with thymuses. The inverse relation to lymphoma latency has already been mentioned.

In experiments preceding those reported here, rats were thymectomized within 1 day of life. This seemed desirable to reduce markedly immunologic competence, but both otive and late peria mortality was so high in this group (approximately 70% at weaning age) that it was decided to thymectomize the rats at 3–4 days. The drop of lymphocyte levels was deeper and more lasting in animals thymectomized within 24 hr of age (Chart 5) than in those thymectomized at 3–4 days (Chart 3).

**Immunologic Deficiency in Thymectomized Rats and Its Correction by Thymus Grafts.** In a small number of rats in which hematologic studies (Charts 3, 4) were carried out, the immunologic capacity was assayed by hemagglutinin production following a single injection of sheep erythrocytes. The technic was rigidly standardized, and the results were read as unknown. In general, the results of 4 tests (Table 2) parallel the hematologic data. They show that thymectomized rats surviving 160 and 200 days are immunologically competent, but not to the extent that rats not operated upon are. Thymus grafts raised the capacity of the rats to produce hemagglutinins.

It should be remembered that these tests were made in animals that survived thymectomy by 160 and 200 days. Data of others indicate that with time there is some recovery from immunologic depression. Many more immunologically deficient rats than indicated in Table 2 may have died earlier of complicating diseases.

**Chart 4.** Blood levels of small lymphocytes in 5 groups of virus-infected, nonleukemic WF rats at termination of the experiment. The figures above the columns indicate the number of rats in the group.

(Figs. 14–22 are electron micrographs of lymphomas arising in thymus grafts on thymectomized rats infected with virus.)

- **Figs. 14, 15** show virus-like particles free in tissue spaces amidst cell processes. $\times 8700$ and $\times 57,000$.
- **Figs. 16, 17.** Numerous virus-like particles in a membrane-lined vesicle. $\times 8700$ and $\times 57,000$.
- **Figs. 18, 19.** Budding of virus on the plasma membrane of a lymphoblast. $\times 8700$ and $\times 57,000$. 

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Viral Leukemia in Thymectomized Rats

Discussion

The high sensitivity of rats to leukemia viruses, as of mice, first indicated by studies of Graffi and Gimmy (8, 9), led to the testing of the rat’s sensitivity to other leukemia viruses (6, 13, 14, 47). Viral lymphoma of the rat, as that of the mouse, is commonly thymic. This suggested systematic investigations in the rat of the presumed noncellular thymic factors that might influence induction of leukemia in mice. The existence of these factors is postulated on the basis of induction of leukemia in host cells by thymic grafts (6) and by restoration of immunologic competence by thymuses grafted in Millipore filters (30, 31, 51). There is no convincing evidence that rats carry a latent leukemia virus, as do many strains of mice. That mice do so is suggested by activation of leukemogenic viruses by radiation (6, 12), immunologic studies of Old et al. (personal communication) and the finding of Pollard with germ-free animals (personal communication). He observed virus particles in thymus in 7 strains of germ-free mice. In 6 strains (ICR, Swiss-Webster, CFW, Balb/C, C3Hf, and C57 BL) he induced leukemia by X-rays. In the 7th strain of germ-free mouse (AKR), he found virus particles in the thymus.

Leukemia is relatively rare in rats, and extensive attempts to develop a high leukemia strain of rats, as was done to develop Ak mice, were unsuccessful (20). However, they incidentally yielded a well-inbred rat strain. W/Fu (20), shown to be homogeneous in transplantation tests (34) and highly sensitive to the passage virus of Gross (49). In our recent extensive studies, no spontaneous leukemias were observed in rats of this strain younger than 12 months, whereas the majority of newborn W/Fu rats succumb within about 3 months to thymic lymphoma when infected within a few days after birth with rat-adapted Gross passage virus. It is our impression that the thymuses of those rats which fail to develop thymic lymphoma have undergone “accidental” thymic involution during the 1st few months of life (23). This in itself suggests that in rats, as in mice, the thymus plays a determining role in the induction of leukemia by virus. This assumption was verified by removing the thymus before

![Chart 5. Total lymphocyte levels in the blood of Sprague-Dawley rats thymectomized within 24 hr after birth and in controls. The curves were drawn by connecting mean values of 5-to-rats examined at the time indicated.](image)

**TABLE 2**

<table>
<thead>
<tr>
<th>No. of thymuses grafted</th>
<th>Rats not infected with virus</th>
<th>Infected rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test 1</td>
<td>Test 2</td>
</tr>
<tr>
<td></td>
<td>(160-170 days old)</td>
<td>(200 days old)</td>
</tr>
<tr>
<td>0</td>
<td>512</td>
<td>256</td>
</tr>
<tr>
<td>512</td>
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<td>2048</td>
<td>4096</td>
<td>256</td>
</tr>
</tbody>
</table>

* Each figure refers to 1 animal. All thymectomies were complete. The grafted thymuses were from neonatal rats. None of the rats in this table had leukemia. Serum of normal rats failed to agglutinate sheep erythrocytes. The age of the rats is approximate.

![Figs. 20, 21. Budding of virus on a process of a stromal cell with adjacent free virus. X 36,500 and X 116,500.](image)

![Figs. 22, 23. Budding of virus at several places on the plasma membrane of an apparently degenerated lymphoblast. X 8700 and X 57,000.](image)

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and at various intervals after virus infection. When the thymus of 1-month-old rats, infected a few days after birth, was removed, many extrathymic lymphomas occurred (24), but when the thymus was removed before virus infection, the development of extrathymic lymphomas, as well as that of myeloid leukemia, was almost completely prevented (23). This pointed to the existence of a thymic factor influential in induction of different types of leukemias.

Grafts of neonatal thymus corrected all 3 deficiencies produced by thymectomy: immunologic incompetence, lymphocytopenia, and unresponsiveness to induction of lymphoma by virus. Following Metcalf's concept (37, 38) of lack of homeostatic control of the thymic mass, some thymectomized rats were given 10 thymuses and others, 5 or only 1 thymus. With 1 exception, correction of these 3 deficiencies was generally enhanced with increased number of grafted thymuses. Regarding restoration of immunologic competence, the data did not show that 10 thymus grafts were better than 5.

The use of 1st generations of 2 highly inbred and genetically unrelated strains of rats, both susceptible to this virus (24), enabled studies to be made on the genetic character of the leukemic cells. They indicated that leukemias in thymectomized rats grafted with thymus originate in lymphoid cells of either the host or the donor, as was shown earlier in mice (42, 45). These transplantation assays were made in F1, and in parental strains. When the tumors took only in the F1 hosts, they were considered to be of the host type. When both types of hosts accepted the lymphoma cells, they were classified as being of the donor type. However, it is possible that these lymphomas were formed by several clones of cells, some of which were of donor and others of host type. (This hypothesis is made on the assumption that nucleic acids carrying histocompatibility information are not incorporated in lymphoid cells.) Precise genetic characterization of the induced lymphoma cells calls for more elaborate assays than that done in the present work.

When the lymphomas could be grafted on neonatal and not on adult rats, we assume that they were altered with respect to their transplantation antigens. When only adult rats were used as recipients and neither type of host accepted the lymphoma cells, we believe that they too were immunogenetically altered. Similarly, regression of grafted tumors in adults was probably due to immunologic modification of the lymphoma cells. Regressions are interpreted as indicative of some antigenic (genetic) modification of lymphoid cells. Failure of takes in either type of host may mean that (a) tumor latency was longer than the period of observation, (b) the tumor cells markedly deviated antigenically from the donor thymic cells, or (c) there was a technical failure. We consider the 2nd possibility to be the most likely one. Our interpretations of regression in the present strain are based on our universal success in grafting many established tumors (20) and on the absence of infection in the tumor grafts. (The media in which the tumor cells were suspended contained penicillin and streptomycin.) Although more precise genetic and immunologic analyses of the induced lymphomas are desirable, especially with the use of thymectomized hosts, the data seem to lead to the conclusion that some were immunologically altered. Many recent studies indicate that immunologic modifications in virus-induced lymphoma cells occur commonly (3, 15, 16, 21, 22, 52).

Miller (41, 45) reported that the age of the thymus rather than the age of the host on which it is grafted appeared to determine sensitivity to leukemia. In the present experiments, single thymus grafts in newborn rats raised the leukemia incidence to 42.9% and, in 2-3-week-old rats, to 31.3%. While this difference may not be significant, the age factor deserves further study.

Although viral multiplication and neoplastic transformation are somewhat related, the 2 are independent processes. The specific role of the thymus lies in its ability to bring about neoplastic transformation. As Metcalf and Wakonig-Vaartaja (39) documented, there are 3 successive types of lymphoid cell population in thymus grafts: donor's, mixed donor's and host's, and host's. Therefore, whether leukemia arises from donor or host cells may depend on the time of leukemic transformation. It is postulated that the thymus secretes some hormone-like substances (25, 36, 48). The cellular source of this substance and its very existence require further documentation. It is possible or even probable that such a substance exists, but it should be recalled that most lymphomas in rats given thymus grafts appear to develop in the grafted thymus. Therefore, the existence of some physical force, remote as this possibility is, needs to be excluded.

Acknowledgments

The rats in the preliminary experiments (Chart 1) were thymectomized by Dr. Antonio Cali. The photographs were taken by Mr. Edward Hajjar. Miss Sandra DeLuca, Mrs. Peggy Moy, and Mr. Harold McQuilla rendered able technical assistance in the animal experimentation. Mrs. Barbara Faucett and Mrs. Elizabeth Osze processed the sections for microscopy. Their assistance is gratefully acknowledged.

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A. Kunii, J. Furth and L. Berwick