The Effect of Cortisone and Thymic Extract in Mouse Viral Leukemia\textsuperscript{1,2}

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Summary

Highly susceptible, newborn F\textsubscript{1} hybrids of AK/Jax × C3Hf/Bi/Gs/L\textsuperscript{2} strain mice, referred to as AK/Z, received Gross Passage A virus i.p., and the leukemogenic process was altered by 2 forms of intervention affecting the target organ, the thymus.

Death from leukemia was delayed but not prevented by monthly courses of cortisone, 1 mg s.c. daily for 3 days. The delay was inversely proportional to the age at which the hormone injections were begun. Delay was possible even when these were begun during the period in which the leukemogenic process was already completed or nearly complete, as judged by previous studies of pathogenesis.

In mice thymectomized at 5 weeks of age, the interrupted leukemogenic process could not be reinitiated by i.p. injections of thymic extract from 1—2 normal mice every 6—7 days, continued for 5 months. Injections of normal mouse thymus in normal newborn AK/Z mice resulted in early lymphocytic lymphoma in 4 of 20 mice.

Introduction

Spontaneous thymic lymphoma in the high leukemic mouse strain AK is similar to the accelerated disease induced by the Passage A virus of Gross in AK/Z mice (7). In both cases autonomous proliferating lymphocytes appear 1st in the thymus, and the fetal thymic or disseminated forms of the disease are prevented or delayed by thymectomy (6). Two experiments involving direct or indirect effects upon the thymus in the course of Passage A virus-induced leukemia were carried out in AK/Z mice to observe alterations in the predictable pattern of the disease.

Materials and Methods

MICE. Mice used were F\textsubscript{1} hybrids of AKR/Jax females and C3Hf/Bi/Gs/L males. Over 70% AK/Z mice develop leukemia spontaneously but with a mean latency of about 300 days, whereas those receiving virus when less than a week of age develop leukemia in over 90% with a mean latency of about 100 days. Young AK/Jax females were mated with Z males, and the offspring, both infected and control, were housed after weaning and numbering by ear punch in groups of 6—8 animals in hanging cages, without segregation by sex. Offspring born to these animals were discarded. Experimental and control mice were fed Purina chow and water ad libitum and observed until death. Moribund animals were killed with ether, and all animals were autopsied. Sections were taken for histologic diagnosis of the type of leukemia whenever possible.

VIRUS. The Passage A virus of Gross was used. Millipore filtrates were prepared from a 20% extract in chilled saline of leukemic tissues (thymus, spleen, and lymph nodes) from several mice with viral leukemia of short latency. Bottled in 1- to 2-ml aliquots, the filtrate was stored at −70°C and unfrozen immediately prior to injection into newborn mice as required. All mice in each experiment were injected from a single filtrate representing the 6th serial viral passage of the virus in our laboratory, since receiving it in its 11th passage from L. Gross in 1957. Mice less than 48 hr of age were given injections i.p. with 0.1 ml of filtrate after 1st traversing the thigh muscles to avoid leakage.

THYMECTOMY. Thymectomy was performed in 5-week-old virus-injected mice under nembutal-ether anesthesia by gently removing the left and right thymuses with fine, curved tweezers through a midline incision to the level of the 3rd rib, after splitting the pretracheal fascia. The incision was closed with a running skin suture. Twenty-five \% of the thymectomies were judged to be incomplete and were eliminated from the data because lymphoma later developed in what appeared to be a thymic remnant.

THYMIC EXTRACT. The thymuses of healthy young mice of various inbred and noninbred low leukemia strains, chiefly C57BL, C57BR, or Harvard Swiss were ground with sand in chilled saline and injected i.p. as a fresh extract or after storage of the extract at −70°C.

EXPERIMENT I. This experiment was on the effect of repeated thymic lymphocyte depletion induced by cortisone. One hundred and four AK/Z mice injected with virus at birth were randomized into 3 main groups with equal proportions of animals of each sex. The 1st group of 24 was given virus alone. The 2nd group of 33 litter mates was given monthly courses of cortisone, 1 mg s.c. daily for 3 days beginning at 59—63 days of age, when the leukemogenic process, on the basis of previous studies (7), could be expected to be completed or nearly completed in most animals, even if not grossly evident. The 3rd group of 47 litter mates was started on monthly courses of cortisone in the same dosage and time sequence during the latent period of the disease. Monthly courses of cortisone were continued for life in both treated groups,
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CORTISONE AND VIRUS INDUCED AK/Z LEUKEMIA

CHART 1. Cumulative mortality due to leukemia in AK/Z mice given i.p. injections of Gross virus when newborn and treated with cortisone s.c. Each circle represents the cumulative percentage of deaths at a particular date. Numerical results above each curve represent % of mice leukemic, e.g., 92% L, with fractions giving in the numerator the number of leukemic mice and in the denominator the total number in each experimental group. Deaths from spontaneous leukemia among control AK/Z mice begin after 200 days, in contrast to the earlier onset of disease in the accelerated form induced by virus injection in the newborn period.

in an attempt to prevent full regeneration of the thymus at any time.

EXPERIMENT II. This experiment was on the effect of thymic extract injections after thymectomy. One hundred AK/Z mice injected at birth with virus were randomized similarly into 4 groups, 1 to be left intact and 3 to be thymectomized. The 1st group of 27 was given virus alone. The 2nd group of 9 thymectomized mice was left as a control for the efficiency of complete thymectomy in preventing or delaying lymphocytic leukemia. The 3rd group of 24 was thymectomized and given an injection every 6-7 days for 5 months of a fresh or frozen saline extract of thymus glands from 1-2 normal mice. The 4th group of 22 was thymectomized and given injections of the same thymic extract heated to 56°C for 1 hr to eliminate the effect of any leukemia virus present in the extracts.

Results

The results of cortisone treatment during the latent period and during the imminent or overt leukemic period are summarized in Chart 1, which shows the cumulative mortality from leukemia in the 3 groups of the 1st experiment. Virus alone resulted in death from leukemia in 92% at a median age of 91 days. Cortisone begun in the early leukemic period moved the median age at leukemic death up to 115 days, an increase of 24 days over the untreated group. Treatment begun in the latent period of disease produced a more marked lengthening, in fact a doubling, of life-span, with the median age at leukemic death now 179 days, an increase of 88 days over the untreated group. However, cortisone is unable to reverse or abort the malignant process due to a potent agent in highly susceptible animals. This is evident from the small differences in ultimate cumulative percentage of death due to leukemia in the 3 groups. The therapeutic effect of cortisone given to mice once malignant cells are already present is brief, but definite, and recalls its action in human leukemia in inducing remissions of variable length.

The results of the 2nd experiment are summarized in Chart 2. In the nonthymectomized group, leukemia was the cause of death in 23 of 27 mice, or 85% at a median age of 95 days. By contrast, in the group of 9 thymectomized mice, leukemia occurred in only 1 of the 9, a generalized disease of undetermined cell type at 515 days. In thymectomized animals given active or heated thymic extract by injection, leukemia accounted for 4 of 24 deaths and 2 of 22 deaths, differences too small to be of any significance when compared with the effect of thymectomy alone.

In previous experiments in our own and other laboratories (3, 6, 11), grafts of normal thymus tissue allow the leukemogenic process interrupted by thymectomy to go to completion in an appreciable proportion of cases, depending upon the genetic susceptibility to leukemic transformation of the cells grafted. By contrast, thymic extract of homologous origin, prepared by a method that preserves its lymphocyte-stimulating activity in baby mice and given at intervals during which lymphocyte-stimulating activity is sustained (9), was completely ineffective in reinstituting the leukemic process.

Chart 3 illustrates that such thymic extracts from normal animals may contain leukemogenic material that is either viral or hormonal. Extracts from normal C57BL mice resulted in 4 early thymic lymphomas when 45 newborn AK/Z mice were given injections of the thymic extract from a single normal mouse and followed for 190-200 days. During this period of observation there were no deaths from leukemia among 54 normal AK/Z controls, which was to be expected, as spontaneous leukemia in these hybrids begins to appear only after 200 days.

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Discussion

The central role of the thymus in lymphocytic viral leukemia induced by Passage A virus is as yet poorly understood. Virus proliferates in a number of tissues, but the effect of thymectomy in preventing or greatly delaying leukemia implies that the thymic lymphocyte is the target cell for transformation. This is correlated with the fact that viral leukemogenesis is most effective in healthy animals, as intercurrent illness associated with thymic atrophy lowers the efficiency of the process. In our laboratory, when endemic pneumonia became established in the Z colony, we were unable to induce a high proportion of deaths from leukemia with the Gross virus until we destroyed the colony and started again with a fresh stock of healthy mice. The relationship between thymic depletion of lymphocytes due to stress (acute starvation), cortisone, or irradiation was examined by Upton and Furth (14) in studying the spontaneous leukemia of AK mice, the high leukemia strain from which the Passage A virus of Gross was derived. Here it was noted that a single episode of cortisone-induced thymic atrophy or depletion, from which the thymus appears fully regenerated in 60 days, was capable of reducing slightly the total proportion of deaths due to spontaneous viral leukemia and, consequently, the total mortality of AK mice observed for 22 months. A single episode of starvation without lasting weight loss also reduced leukemia in a similar degree. A greater reduction was achieved by cortisone given in 3 courses over a 14-week period. Even more striking results were obtained by Wooley and Peters (15), who continued courses of cortisone in AK mice throughout life and therefore achieved a reduction in deaths due to spontaneous leukemia nearly comparable to that produced by thymectomy of AK mice in the hands of McEndy et al. (8).

By contrast, cortisone-induced thymic depletion of lymphocytes in repeated courses throughout life is unable to prevent, although it may delay, death from leukemia when the process is initiated by a highly potent virus in very susceptible animals. The length of the delay is inversely related to the age at which cortisone is begun, being most marked in young mice and slightest in older mice, when the leukemogenic process is nearly completed or already complete. The mechanism of action of cortisone upon either the spontaneous viral or the filtrate-induced viral leukemia can only be postulated, but dispersion of thymic lymphocytes from the intimate contact with thymic reticuloendothelial influence appears to render them no longer susceptible to the leukemogenic effect of virus. In addition, cortisone,
metabolized in vivo to its cytotolytic form hydrocortisone (13), by inducing lysis of both normal lymphocytes and lymphoma cells (2), may destroy those already leukemic and diminish the number of those susceptible to leukemogenesis in the thymus.

The definite but as yet unexplained influence of lymphocyte environment upon viral leukemogenesis can be studied more critically after thymectomy, which interrupts the process in an even more decisive manner than cortisone-induced thymic atrophy. The fact that some cells originating in the thymus seed the lymphoid organs of the body appears reasonably well established (10, 12). Such cells seem to have undergone a change or acquired an environment that renders them no longer susceptible to viral leukemic transformation. What role, if any, is played by the secretions of the thymic reticuloendothelium in the process is also still undetermined. Lymphocytes of virus-infected thymectomized hybrid mice grafted with thymus of parental pure strain origin are attracted to the graft and can there undergo malignant transformation (6, 11). This implies that cells populating the lymphoid tissues, upon migrating back into contact with the reticuloendothelial secretory portion of the thymus gland and proliferating there, resume whatever condition it was that renders them the target cells of the leukemia virus. Not a particular cell per se, but a cell in a particular environment, appears to be the critical combination. Our attempt to supply the nonthymic lymphoid cells with 1 factor in that environment, the secretion of the thymic reticuloendothelium, proved inadequate to create the conditions necessary for transformation. Access to a continual supply of thymic secretion derived from thymic tissue in Millipore capsules implanted in virus-injected thymectomized mice or rats has been similarly unsuccessful in a number of laboratories (4, 10). Unfortunately, it is clear that neither intermittent injections nor a single graft in a Millipore capsule has so far supplied necessary factors in the concentration and combination available to the actively proliferating immature lymphocytes in immediate physical proximity to the thymic reticuloendothelium.

The stimulus to proliferation derived from contact with thymic reticuloendothelial tissue may be an important factor in Gross mouse viral leukemogenesis, and the rapidly dividing immature lymphocyte may well be the only cell susceptible to lymphocytic leukemic transformation. In this case, attempts to re-create this milieu in the thymectomized animal, without permitting direct cell-to-cell contact, are understandably difficult. However, since, Millipore capsule grafts of thymic tissue can alter the immunologic behavior of nonthymic lymphoid cells of mice with respect to skin grafts, viral infection, and primary immune response to sheep hemolysins (5), it is possible that the factors necessary for lymphoid cell proliferation and for viral leukemic transformation could be supplied by more extensive or repeated grafts, or more intensive courses of homologous or heterologous thymic extract.

These experiments serve to underline the complexity of the mouse viral leukemogenic process, with the varying influence of genetic factors, age, virus, cell, and cell environment. It is reasonable to expect that a similar complexity will be found to exist in the human counterpart to this disease.

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References

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