Thymolytic and Adrenal Cortical Responses to the LDH-Elevating Virus[1] (36109)

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(Introduced by V. Riley)

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Accumulating evidence suggests that potentially leukemogenic viruses may be present in mice and other animals, including man, as silent inhabitants, and that the leukemogenic potentials of these viruses may be evoked by one or several chemical, physical or physiological host factors. Host circumstances which might affect the leukemogenic properties of resident viruses include humoral factors associated with the thymus, which in turn are influenced by the adrenal cortical hormones. Inasmuch as the thymus and thymus-dependent lymphocytes are also involved in cell-mediated immune responses, it seems appropriate to examine the responses of the thymus, the lymphatic organs, and the adrenal cortex, following controlled infections with oncogenic and non-oncogenic viruses.

The basic biological characteristics of the benign, non-oncogenic, lactate dehydrogenase-elevating virus (LDH-virus) have been described (1–11). This virus is physically associated with most transplanted mouse tumors, and has been found to inhibit the leukemia induced by the Friend virus (3), mammary tumors induced by the Bittner virus (4), and the "spontaneous" leukemia of AKR strain mice (1). Following inoculation of mice with the LDH-virus, there is a rapid increase in virus titer, reaching 10^{10} ID_{50}/ml of plasma, usually within 12-24 hr (1). Of special significance is the persistence of a viremia throughout the normal life span of the infected mouse. This chronic virus titer exhibits 10^4–10^6 infectious units per ml of plasma, but induces no overt pathological symptoms. An elevation of several plasma enzymes accompanies the viremia, the most conspicuous being lactate dehydrogenase (LDH), which attains a tenfold elevation within 72–96 hr following inoculation of the virus. This new enzyme equilibrium level is maintained during the lifespan of the animal and may be considered a virus-induced biochemical lesion.

A lymphocytopenia is observed 24–48 hr following LDH-virus inoculation. This in turn is followed by a lymphocytosis, which becomes evident about the fourth day after inoculation. Functional alterations of the reticuloendothelial system are indicated by a temporarily impaired carbon clearance, which returns to normal by the sixth day after inoculation (1, 2, 5). In addition, there is an inhibition of thymus-dependent cell-mediated immunity (6, 7) as well as an enhancement of thymus-independent humoral immunity (6).

Following are the results of experiments designed to examine the involvement of the thymus, spleen, lymph nodes and the adrenal cortical hormones in mice infected with the LDH-virus.

Material and Methods. Mice. Hybrid (C57BL/6 × A)F_1 (BAF) female mice were used in these studies. These were obtained from commercial breeders at approximately five weeks of age. Upon arrival in our laboratory they were randomly segre-

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gated into groups of ten, and stored in standard plastic holding cages. These cage groupings, or subdivisions into groups of five mice each, were maintained throughout the experimental period. The experiments were begun when the animals were approximately ten weeks of age, weighing 17 to 20 g.

Animal housing. All mice were housed within specially designed, enclosed ventilated shelves with laminar air flow which assured environmental protection against outside or intercolony infection and cross-contamination with various infectious agents (12). These housing facilities also minimized uncontrolled stress-inducing stimuli, which is of obvious significance in studies involving adrenal cortical responses.

The lactate dehydrogenase-elevating virus. (LDH-virus). Pooled mouse plasma having an infectious LDH-virus titer of approximately 10^{10} ID_{50} units per ml was diluted 1/100 and inoculated intraperitoneally in a 0.1 ml volume. Characterization of the LDH-virus and a description of assay methods have been previously reported (1).

Organ weights. The thymus weight responses of intact mice to infection with the LDH-virus were compared with those of animals adrenalectomized 24 hr prior to inoculation with the virus. Non-infected intact and adrenalectomized mice were sham-injected with saline and used as controls. The LDH-virus-infected groups were inoculated with 10^7 ID_{50} infectious units per mouse. All groups except the adrenalectomized sham-injected controls were autopsied at 24, 48 and 96 hr after inoculation or sham injection. These intervals were chosen to coincide with the early peak in LDH-virus titers, and with lymphocytopenia which has been shown to occur following inoculation with the LDH-virus (1). The adrenalectomized sham-injected mice were autopsied at 24 and 96 hr after sham injection. At autopsy the weights of the thymus, spleen and pooled inguinal and axillary lymph nodes were determined for each animal.

Results. Thymus involution. As shown in Fig. 1, infection with the LDH-virus induced a statistically significant thymus involution in

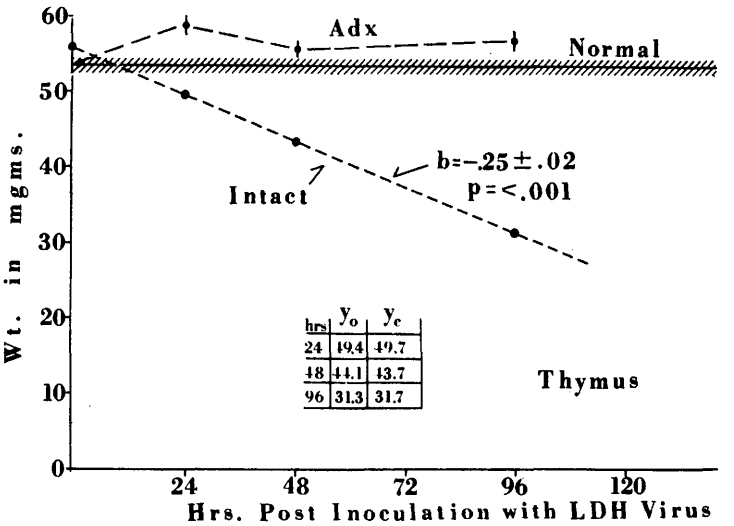

FIG. 1. Thymus weights of intact and adrenalectomized mice, determined at 24, 48 and 96 hr following inoculation with LDH-virus.

The thymus regression line of the intact, virus-infected mice was fitted by the method of least-squares, and was statistically significant, as indicated by a p value of less than .001. The slope, b, indicates that following virus infection, the mean thymus weight of these mice decreased at the rate of 0.25 mg/hr. Y_o = observed mean thymus weight. Y_c = values calculated by least-squares method.

The shaded bar represents the average values and standard error of the mean thymus weight of the non-infected, intact normal controls.

intact mice. In contrast, the adrenalectomized virus-infected mice exhibited a slight increase in thymus weight, and the sham-injected intact mice showed an insignificant thymus weight decrease. These results support the working hypothesis that the LDH-virus induced an increase in circulating adrenal cortical hormones, which in turn mediated the thymus involution.

As indicated in Table I, the LDH-virus titer was essentially the same in the intact and adrenalectomized mice; thus, the observed differences in thymic response to infection with this virus were not caused by a differential virus titer in the two groups.

TABLE I. Comparison of LDH-Virus Titer in Intact and Adrenalectomized Mice.

Hours following virus infection	Virus titer ($\log_{10} ID_{50}$/ml plasma)	
	Intact mice	Adrenalectomized mice
24	10.0	10.0
48	9.5	9.5
96	8.5[a]	7.5[a]

[a] Difference is not statistically significant.

Response of lymph nodes and spleen. In contrast to the observed thymus involution, progressive weight increases of the peripheral nodes and spleen were observed in the intact, as well as in the adrenalectomized virus-infected animals, as shown in Fig. 2. Ninety-six hours following virus inoculation, the lymph nodes and spleen had increased in weight by approximately 57% and 29%, respectively, in the intact mice; and by 100% and 58%, respectively, in the adrenalectomized animals. These weight increases were all statistically significant. It may be of interest that the organ weights of the virus-infected animals were significantly greater in the adrenalectomized mice than in the intact animals.

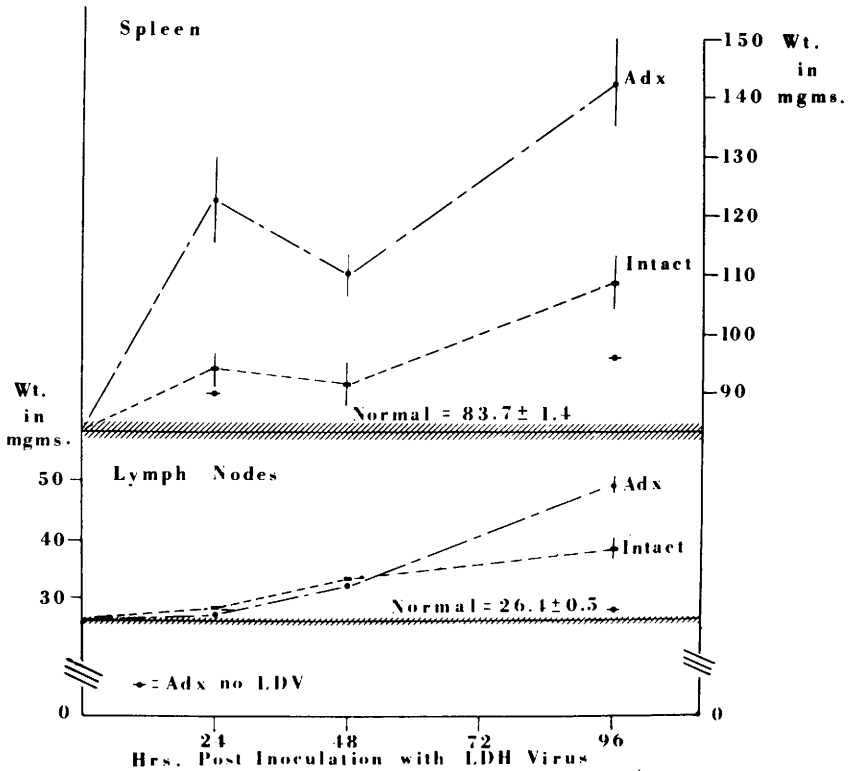

FIG. 2. Spleen and lymph node weights of intact and adrenalectomized mice at 24, 48 and 96 hr following inoculation with the LDH-virus. Vertical and shaded bars represent the standard deviations of the mean organ weights.

Further analysis of the weight responses of the spleen and lymph nodes showed that a significant increase in spleen weight was evident within 24 hr after virus inoculation, in both the adrenalectomized and intact animals. However, the weight increase of the lymph nodes was more gradual, and not clearly evident until 96 hr following virus inoculation.

In the non-infected adrenalectomized animals, a small increase in spleen weight was observed, which, however, was of doubtful statistical significance. The weight of the lymph nodes in these mice failed to increase. Thus, while the LDH-virus infection induced a weight increase of the lymphatic organs in both the intact and adrenalectomized animals, the virus-induced weight increase, particularly of the spleen, was accentuated by adrenalectomy.

Discussion. Infection of intact mice with the LDH-virus induced a statistically significant thymus involution which was linear with time for the first 96 hr (8). The lack of thymus regression in adrenalectomized mice following virus infection supports the hypothesis that the thymus involution in the intact virus-infected mice was mediated by an increase in circulating adrenal cortical hormones.

Earlier studies have demonstrated that the thymus, lymph nodes and spleen are partially under the control of the adrenal cortical hormones. Adrenalectomy was found to induce an enlargement of these organs, which was associated with an increase in the cellularity of the lymphoid tissues (13–16). When such adrenalectomized animals were injected with small doses of adrenal cortical hormones, this enlargement of the spleen and lymph nodes was not observed. If the hormone dose was large enough however, a reduction in the size of the lymphatic organs occurred. Further studies indicated that a quantitative relationship exists between the exogenous hormone dose and the decrease in lymphatic organ weights in adrenalectomized mice (15–17).

The lymphocytolysis occuring in animals treated with adrenal cortical hormones has been attributed to a destruction of the thymus-dependent lymphocytes. An inhibition of mitosis and differentiation during lymphocytopoiesis also occurs (15–16). The thymus is more sensitive than either the spleen or lymph nodes to the effects of the adrenal cortical hormones. The relationship between the regression of the thymus and the hormone dose is so precise that thymus involution has been utilized as a convenient, indirect bioassy for adrenal cortical hormones (15–17).

Thymus regression has also been employed as an indicator of increases in adrenal cortical activity induced by stress (16–17). Following subjection of animals to various types of stressing stimuli, an involution of the thymus, but not of the lymph nodes and spleen, occurs (18). This thymus involution fails to occur in adrenalectomized animals (16). The failure of the sham-injected, intact mice in these experiments to exhibit a significant thymus involution eliminates the possibility that simple stress due to manipulation might account for the thymolytic effects observed following LDH-virus inoculation.

The lymphocytopenia which has been observed during the acute phase of LDH-virus infection (1) is consistent with the observed thymolytic effects of the virus, since a depletion of thymus-dependent blood lymphocytes is also a normal response to an increase in circulating adrenal cortical steroids.

The significant weight increases of the spleen and lymph nodes observed following LDH-virus infection of intact mice are not consistent with previous observations of spleen and lymph node weight decreases following the administration of adrenal cortical steroids (15). However, two effects of LDH-virus infection upon the spleen and lymph nodes have been described. In light and electron microscopic studies, an early and continuous immunoblast proliferation was observed in the thymus-independent regions, concomitant with a destruction of small lymphocytes in the thymus-dependent regions (9–11). The latter observation is consistent with adrenal cortical activation by the LDH-virus.

The virus-induced proliferation in the thymus-independent regions of the spleen and lymph nodes (9, 10) probably accounts for the weight increases observed in these organs

following LDH-virus infection. The accentuation of these virus-induced organ weight increases in the adrenalectomized mice, in which adrenal hormone-mediated destruction of thymus-dependent cells cannot occur, further supports the data indicating that the LDH-virus induces an activation of the adrenal cortex.

Summary. Following infection of intact mice with the LDH-virus, a significant thymus involution was observed. Since there was no thymus involution in adrenalectomized mice infected with the virus, a virus-induced adrenal cortical hormone increase or activation is postulated. The observation of an increase in spleen and lymph node weight in both intact and adrenalectomized LDH-virus infected mice may result from a virus-induced proliferation of thymus-independent lymphatic tissue, which is unaffected by the adrenal cortical hormones.

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