

Influence of Prednisolone on Moloney Leukemogenic Virus in BALB/c Mice¹

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SUMMARY

Long-term administration of prednisolone to BALB/c mice alters the reactivity of their lymphoid tissue to the oncogenic effects of Moloney leukemia virus. Solid lymphosarcoma or granulocytic leukemia is produced in a significant number of the treated mice. The alteration in disease response may be attributed to a prednisolone-induced simulated thymectomy.

INTRODUCTION

In susceptible animals, the thymus is the primary target tissue of neoplastic transformation by the murine lymphocyte leukemia viruses (26). It is well established that thymectomy decreases the incidence of spontaneous and experimental virus-induced murine lymphoid tumors while significantly increasing the incidence of myeloid leukemia and reticulum cell sarcoma (14, 16, 17, 22, 24). It is therefore suggested that if the thymus, which is most susceptible to the influence of lymphocytic leukemia viruses, is removed, the increased longevity of the mouse allows other neoplasms of the reticular system to become manifest. In addition, the thymus may exert a regulatory influence over myeloid tissue (20, 25).

Thymic atrophy can be chemically induced by the action of various corticosteroid preparations (5, 7, 9, 10, 32).

This paper reports on our efforts to influence the outcome of MLV³ infection in BALB/c mice by the long-term administration of prednisolone. Granulocytic leukemia and solid lymphoid tumors developed in a significant number of mice.

MATERIALS AND METHODS

Mice. Newborn BALB/c mice, bred and maintained at Microbiological Associates, Inc., Walkersville, Md., were used.

All mice were fed Purina laboratory chow and water *ad libitum*. Mice were not separated according to sex, but were randomly divided into 6 groups: (a) high-dose steroid plus virus, (b) low-dose steroid plus virus, (c) virus alone, (d) high-dose steroid alone, (e) low-dose steroid alone, and (f) environmental control. All mice were palpated for splenomegaly twice a week beginning at 28 days of age. Test mice were sacrificed when they developed 4+ enlarged spleens. Moribund mice, regardless of splenomegaly, were also sacrificed and examined for evidence of leukemia. Control mice were sacrificed at corresponding times for purposes of comparison.

Adrenalcorticosteroid Treatment. Steroid treatment was begun less than 24 hr after birth with either 0.025 or 0.05 mg of prednisolone acetate s.c. (Rugby Laboratories, Inc.) and continued twice a week until the mice were sacrificed.

Virus. MLV (Pfizer Lot 3042-278) was diluted 1:100 with phosphate-buffered saline, and 0.1 ml was given by inoculation either i.p. or i.v. The virus inoculations into steroid-treated and virus control mice were made 7, 14, 28, 42, or 56 days after birth. Concomitantly, other groups of steroid-treated mice received 0.1 ml of phosphate-buffered saline.

Preparation of Tissues. Prior to sacrifice, a peripheral blood smear was made from a drop of tail vein blood. Mice were sacrificed by cervical dislocation. Tissues were fixed in either Zenker formol or 10% buffered formalin and stained with hematoxylin and eosin. Blood films were stained with Giemsa.

Criteria for Diagnosis. The classification of reticular neoplastic diseases presented by Dunn (11) was used as a guide in arriving at a diagnosis. Evaluations were made on the basis of the combined gross, microscopic, and peripheral blood findings.

RESULTS

Our results show that long-term administration of prednisolone to BALB/c mice altered their response to infection with MLV. In the prednisolone plus MLV groups, a total of 131 mice were found to have leukemia or lymphoma. Of these 131, 19 mice (14.5%) had neoplasms other than lymphocytic leukemia. From one solid lymphosarcoma, a new lymphosarcoma-producing virus has been isolated (1, 2). Prednisolone protected mice inoculated at 7 or 14 days of age by significantly increasing the latent period to the development of lymphocytic leukemia.

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A total of 495 newborn BALB/c mice were used at the onset of this experiment. Of these, 39 died prior to being palpated at 28 days of age. Another 31 died during the course of the experiment and were not available for necropsy. Termination of the experiment after 1 year found 83 mice still alive. The data reported comprise the information from the remaining 342 mice.

High-Dose Prednisolone plus MLV. Of 85 mice examined, lymphocytic leukemia occurred in 50. Granulocytic leukemia was seen in 4 cases, 3 of which were chloroleukemia (Table 1). Six mice had lymphosarcoma; 1 had stem cell leukemia, and reticulum cell sarcoma was seen in another. Some degree of lymphatic hyperplasia was observed in 9 mice. Fourteen mice showed no evidence of leukemia. Eight of the 14 nonleukemic mice were runted. One nonleukemic mouse had a mammary adenocarcinoma.

The majority of peripheral blood smears from the leukemic mice showed an apparent leukocytosis. The 4 mice with granulocytic leukemia had white blood cell differentials of essentially all myeloid forms.

Mice with lymphocytic leukemia were evenly divided between those with a predominance of lymphoid cells in their peripheral blood smears and those with predominantly myeloid forms.

Histologically, there were no changes in the adrenal glands or lymphoid tissue directly attributable to the action of prednisolone.

Low-Dose Prednisolone plus MLV. Seventy-eight mice were examined, of which 60 mice had lymphocytic leukemia. Of the remaining mice, granulocytic leukemia was found in 2 animals. Lymphosarcoma occurred in 6 cases, 2 of which were solitary thymic neoplasms. One mouse had a mixed lymphocytic-granulocytic leukemia. Lymphatic hyperplasia was observed in 7 mice. Two mice showed no evidence of neoplasia.

Here, as in the high-dose prednisolone plus virus group,

there were no changes in the adrenal glands or lymphoid tissue attributable to the action of prednisolone. The peripheral blood smears were also similar to the previous group.

MLV Alone, No Prednisolone. The latent period to the development of lymphocytic leukemia in the various virus control groups is given in Table 2. A lymphocytic leukemia pattern consistent with that reported previously (24) for MLV was observed in 83 of the 92 mice examined. No granulocytic leukemia or reticulum cell neoplasms were found. A solitary lymphosarcoma arising from a cervical lymph node rather than a diffuse circulating leukemia was found in 1 mouse.

Peripheral blood smears generally showed an increased number and predominance of mature lymphoid cells. In no instance did granulocytes predominate.

High-Dose Prednisolone, No Virus, and Low-Dose Prednisolone, No Virus. No tumors or leukemia were found. Peripheral blood smears were normal with respect to the number and differential count of white blood cells.

The adrenal glands were histologically normal for the ages and strain of mice examined. The thymuses and lymph nodes showed no sign of lymphocytic depletion.

Environmental Controls. Environmental control mice exhibited no gross or histological deviation from normal.

Comparison of Latent Period by Treatment Groups. Significant differences in the latent period to the development of lymphocytic leukemia were found only in the 7-day and 14-day inoculation groups. In both the 7- and 14-day groups, with either i.p. or i.v. inoculation, the high-dose prednisolone plus MLV mice had a significantly longer latent period than did those mice receiving MLV alone. In the 7-day i.p. and 14-day i.p. groups, the high-dose prednisolone plus MLV mice also had a significantly longer latent period than the low-dose prednisolone plus MLV group. In the 7-day i.v. and the 14-day i.v. groups, the low-dose prednisolone plus MLV mice had a significantly longer latent period than the virus

Table 1

Incidence, age at occurrence, and route of inoculation of reticular neoplasms other than lymphocytic leukemia

Incidence in each case refers to the appearance of a single tumor in the specified number of days.

Treatment group	Granulocytic leukemia			Lymphosarcoma			Reticulum cell sarcoma			Stem cell leukemia			Mixed granulocytic lymphocytic leukemia		
	Age (days) at occurrence	Age (days) at which dose was given	Route of injection	Age (days) at occurrence	Age (days) at which dose was given	Route of injection	Age (days) at occurrence	Age (days) at which dose was given	Route of injection	Age (days) at occurrence	Age (days) at which dose was given	Route of injection	Age (days) at occurrence	Age (days) at which dose was given	Route of injection
High-dose prednisolone plus MLV	189	7	i.p.	174	7	i.v.	357	56	i.p.	290	14	i.v.			
	160 ^a	7	i.v.	205	14	i.v.									
	189 ^a	7	i.v.	339	14	i.p.									
	165 ^a	28	i.p.	93 ^b	28	i.p.									
			297	28	i.p.										
			282	42	i.p.										
Low-dose prednisolone plus MLV	273	28	i.p.										182	14	i.v.
	216	42	i.p.	105	14	i.p.									
				147	14	i.p.									
				105	14	i.v.									
				182	14	i.v.									
				268	42	i.v.									
			253	56	i.v.										
MLV alone				199	56	i.p.									

^aChloroleukemia.

^bOriginal tumor from which the lymphosarcoma virus was isolated.

Table 2
Comparison of latent period (days^a) to the development of lymphocytic leukemia

Age at inoculation (days)	Route	MLV alone (A)	Low-dose prednisolone plus MLV (B)	High-dose prednisolone plus MLV (C)	Significance of ordered mean comparisons in an unweighted means analysis ^b
7	i.p.	112 (86-219)	107 (63-182)	170 (93-226)	C <u>AB</u> , $p < 0.05$
	i.v.	100 (77-154)	134 (77-220)	157 (57-240)	A <u>BC</u> , $p < 0.05$
14	i.p.	117 (86-219)	123 (84-175)	215 (105-270)	C <u>AB</u> , $p < 0.05$
	i.v.	108 (84-147)	154 (105-226)	184 (63-269)	A <u>BC</u> , $p < 0.05$
28	i.p.	145 (98-259)	143 (105-168)	172 (126-207)	
	i.v.	138 (86-231)	162 (105-224)	119 ^c	
42	i.p.	149 (125-257)	195 (118-301)	204 (111-261)	
	i.v.	203 (118-299)	115 (105-125)	158 (91-218)	
56	i.p.	186 (147-341)	190 (77-332)	170 (77-240)	
	i.v.	193 (102-275)	231 (217-245)	208 (199-217)	

^aMean and range.

^bThe underlined groups are not significantly different from each other, but are significantly different from the nonunderlined group at the 5% level.

^cOne observation.

control mice. The comparison of latent period for the various groups is given in Table 2.⁴

DISCUSSION

Our results indicate that the administration of a potent corticosteroid, prednisolone, to BALB/c mice greatly alters the biological response of the treated mice to subsequent challenge with MLV. In the prednisolone-treated groups, some mice developed granulocytic leukemia and others developed solid lymphoid neoplasms. From one of the solid lymphoid tumors, a new lymphosarcoma-producing virus has been isolated (1, 2).

Thymic atrophy can be chemically induced by the action of adrenocorticotrophic hormone (5), cortisone (32), prednisolone (7), and other related adrenocortical hormones (9, 10). Prednisolone is 3 to 4 times more active than cortisol in inducing thymic involution in rats (13). A single large dose of prednisolone in newborn rats produces a functional thymectomy (6). Antibody response in these animals is selectively impaired and resembles the deficit found in surgically thymectomized rats and mice (4).

Surgical thymectomy decreases the incidence of spontaneous and experimental virus-induced murine lymphoid tumors while increasing the incidence of myeloid leukemia and reticulum cell sarcoma (14, 16, 17, 22, 24).

It has long been known that corticosteroids delay or decrease the incidence of spontaneous (22, 33, 35), radiation-induced (18, 33), and experimental virus-induced (21)

lymphocytic neoplasms in mice. In addition, intrinsic function of the adrenal glands may differ in high- and low-leukemia strains of mice. There is evidence of adrenal hypofunction in the high-leukemia strain of AKR mice (23). Adrenalectomy does, in fact, increase the incidence of lymphocytic leukemia in mice (18, 19).

The potentiating effect of steroid hormones on the production of murine lymphoid tumors has also been reported (15, 29). Further, intermittent cortisone therapy was implicated in the development of myeloid leukemia in Swiss mice (34).

The pathogenesis of the lymphocytic leukemia induced by MLV has been described in mice (12). Striking alterations in this disease process have been made by thymectomy (22) or administration of antilymphocyte serum (3). With both of these treatments, which depress immune responsiveness, the incidence of lymphocytic leukemia decreased and reticulum cell sarcomas increased. Corticosteroids also depress immune responses, and are capable of depleting lymphocytes from the thymus (7). The influence of corticosteroids on experimental viral leukemogenesis has been studied with other oncogenic viruses (8, 21, 28, 30), but not with MLV.

Chronic suppression of the thymus with prednisolone produced a "functional thymectomy" in a small percentage of our mice. This concept of a functional thymectomy is supported by the appearance of 6 cases of granulocytic leukemia, 1 mixed granulocytic-lymphocytic leukemia, and 1 reticulum cell sarcoma in the prednisolone-treated groups inoculated with MLV. In addition, there were 11 cases of solid lymphosarcomas not involving the thymus. Although solid lymphoid tumor production has been reported with the murine lymphocytic leukemia viruses, it is extremely rare in mice receiving MLV (J. B. Moloney, National Cancer Institute, Bethesda, Md., personal communication). Four of these lymphosarcomas were frozen and later passaged in BALB/c

⁴Statistical analyses were performed by Foster D. McClure, Microbiological Associates, Inc., Walkersville, Md.

mice. From one of these lymphosarcomas, a virus producing lymphosarcoma has been isolated (1). Further work on this lymphosarcoma virus is reported in another paper (2).

These results indicate that long-term treatment with prednisolone in BALB/c mice strikingly alters the reactivity of their lymphoid tissue to infection with MLV. Lymphoid organs are not involuted by this steroid therapy; therefore, physical removal of the target tissue does not seem to account for the changes. Mice were not sacrificed early in the experiment to study initial steroid effects on lymphoid tissue. Although no late histological effects of prednisolone treatment were seen, critical effects may still have occurred. Significant alterations in thymus-dependent processes may be induced by antilymphocyte serum without any detectable histological change in the thymus (31). Prednisolone did not have a direct effect on viral replication as measured by a mouse spleen assay.⁵

If the thymus does exert a regulatory influence over myeloid tissue (20), then inhibition of a thymic humoral factor which suppresses myeloid tissue could make this tissue more susceptible to the oncogenic effects of MLV. Alternatively, prednisolone may make thymic lymphocytes more resistant to neoplastic transformation by MLV, thereby allowing the virus to transform the less susceptible cells of the myeloid series. If prednisolone acts directly on the thymocyte population to increase their resistance to transformation, and if the thymus is affected to a greater degree by the hormone than are the lymph nodes (27), then possibly the peripheral lymphocytes acted, in this situation, as did myeloid cells, as a secondary target tissue for the MLV to give solid lymphoid tumors. The immunosuppressive effects of prednisolone may also modify cell-mediated immune responses such that transformed cells in the lymph nodes which would be eliminated in normal mice are allowed to proliferate.

Use of prednisolone is clearly another means for modifying the biological response of BALB/c mice to the induction of disease by MLV.

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⁵ Assay performed by Dr. G. J. Spahn, Microbiological Associates, Inc., Walkersville, Md.

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