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Acceleration by Thymosin of the Development of Resistance to Murine Sarcoma Virus-Induced Tumor in Mice*

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Abstract. The development of resistance to progressive tumor growth and the effect of a thymic extract upon this developmental process were examined in CBA/Wh mice. Inoculating mice of various ages with murine sarcoma virus (Moloney) permitted the assessment of the period of time postnatally at which the animals developed a threshold level of resistance to progressive tumor growth. Resistance in CBA/Wh mice, at the virus dose used, was first detected at approximately two weeks of age and was completely developed at five weeks.

Thymosin, a soluble calf thymic fraction, when administered to neonatal mice beginning on the second day of life, significantly accelerated the rate of development of resistance to progressive tumor growth. The data suggest a humoral role for the thymus in the development of tumor immunity, and that the normal development of cell-mediated immunological competence can be accelerated through the use of thymus-derived extracts.

Murine sarcoma virus (Moloney) characteristically induces sarcomas at the site of inoculation after a very short latency period.[1] Although adult and newborn mice are equally susceptible to the induction of tumors by high doses of the virus, mature animals are more resistant to progressive tumor growth than are newborn mice.[2] In newborn and in immunologically deficient mice, the tumor continues to grow and ultimately causes the death of the host. In contrast, in immunologically competent mice, the tumor generally regresses within 2–3 weeks after its initial appearance.

The induction and rate of growth of a tumor after a challenge by an oncogenic virus is a complex phenomenon and the degree of host resistance depends upon a number of factors. Genetic factors have been implicated in affecting both susceptibility to,[3] and prognosis of[2] infection by oncogenic viruses. Factors such as body weight,[4,5] gnotobiotic status,[6] and the production of interferon[7,8] also modify the consequences of viral infections. However, a major factor determining the final outcome of infection by oncogenic viruses appears to be the immunological competence of the host. Animals subjected to procedures which reduce immunological competence show an increase in both their susceptibility to virus-induced tumors[9,10] and the incidence of progressive tumor growth.[2,9]

Furthermore, tumor regression appears to be associated with an immune response directed against tumor-specific antigens.[11,12]

A normally functioning thymus gland is necessary for both the development and expression of the immune response, particularly with regard to its cell-mediated component.[13] Although the exact mechanism by which the thymus exerts this influence on the immune system is still unclear, it appears that the thymus functions as both a donor of cells to the peripheral lymphoid system and as an endocrine gland.[14,15]

The isolation and partial purification of thymosin,[16] a soluble thymus-derived lymphocytopoietic factor, has contributed to the further elucidation of the humoral role of the thymus. When administered to neonatally thymectomized animals, thymosin decreases the incidence of wasting disease and death[17] and restores cell-mediated immunity as measured by the allograft reaction[18] and the graft-versus-host response.[19]

The role of cell-mediated immunity in host resistance to oncogenic processes and the established role of thymosin in the enhancement of cell-mediated immunity in neonatal mice suggested that the administration of thymosin to mice prior to inoculation with murine sarcoma virus (Moloney) might augment their resistance to the consequences of infection with an oncogenic virus. A positive answer to this question is provided by the experiments reported in this communication.

Materials and Methods. CBA/Wh[20] mice bred in our own colony were used in all experiments. Animals were maintained on standard food pellets (Purina Chow) and water *ad libitum*. Pregnant mothers received tap water containing 1% tetracycline until their litters were weaned. Murine sarcoma virus (Moloney), Lot no. 144-R, used in this study was a gift from Dr. J. B. Moloney of the National Cancer Institute. All virus inocula consisted of 0.1 ml of a 1:10 dilution of Moloney strain in phosphate-buffered saline by the subcutaneous-intramuscular route (thigh muscle of one hind limb) as described by Blumenshein and Moloney.[21] Only those mice surviving the first week after inoculation are included in the data presented. Animals were inoculated at the ages indicated in the results, with a latitude of 2 days.

Newborn mice were divided into groups which varied in number from 13 to 43 animals. Groups were either used as untreated controls or were injected with either saline, calf thymosin (*fraction 3*), or a comparable calf liver or spleen fraction, each prepared according to the procedure of Goldstein *et al.*[16] Treated mice received six injections over a 14 day period, beginning on the second day of life. During the first week, three injections of 0.5 mg (as protein) of either thymosin or a liver or a spleen preparation were administered intraperitoneally in 0.05-ml aliquots of 0.15 M NaCl; during the second week, three injections of each fraction (1.0 mg protein in 0.1 ml of 0.15 M NaCl) were given subcutaneously. At 2 weeks of age, all animals were challenged with murine sarcoma virus (Moloney).

Results. Irrespective of the age of the mice at the time of virus inoculation or of the treatment they received prior to virus inoculation, the efficiency of tumor induction and the latency periods did not differ significantly among the various groups of animals (Table 1). However, although the mice of different age groups were uniformly susceptible to tumor induction, they were not equally susceptible to progressive growth of the tumors (Table 1). Figure 1 shows plotted data illustrating the development of resistance to progressive tumor growth in CBA/Wh mice inoculated at varying ages with murine sarcoma virus

TABLE 1. *Development of resistance to progressive growth of murine sarcoma virus (Moloney)-induced tumor in control and in thymosin treated CBA/Wh mice.*

Treatment	Age at inoculation (weeks)	Tumor incidence*	Mean latency period in days ± standard deviations	No. mice surviving 60 days/no. developing tumors
(A) Untreated	Newborn	11/13 (85%)†	6.0 ± 2.0	0/13 (0%)
(B) Untreated	1	17/17 (100%)	6.9 ± 1.7	0/17 (0%)
(C) Saline	2	43/43 (100%)	6.1 ± 1.5	7/43 (16%)§
(D) Liver extract‡	2	23/23 (100%)	6.7 ± 1.0	4/23 (17%)§
(E) Spleen extract‡	2	18/19 (95%)	6.0 ± 0.7	4/18 (22%)§
(F) Thymosin‡	2	36/36 (100%)	6.8 ± 1.3	14/36 (39%)§
(G) Untreated	3	25/25 (100%)	6.0 ± 0.9	14/25 (56%)§
(H) Untreated	5	21/21 (100%)	5.9 ± 0.5	21/21 (100%)

* No. of mice developing tumors/no. of mice inoculated with murine sarcoma virus.

† Although two animals did not develop palpable tumors, they died at 8 and 11 days postinoculation.

‡ First week: 0.5 mg protein of either calf thymosin (fraction 3), calf liver (fraction 3) or calf spleen (fraction 3) three times a week; second week: 1.0 mg protein, three times a week.

§ For statistical analysis, see text. Groups (C), (D), and (E) differ significantly from group (G). Groups (F) and (G) are not significantly different from one another.

(Moloney). Resistance to progressive tumor growth was not seen in mice inoculated either at birth or at 1 week of age, but began to develop between 1 and 2 weeks postnatally. By 5 weeks of age, the development of resistance was complete.

Figure 2 shows a comparison of the 60-day survival rate in CBA/Wh mice challenged with Moloney strain virus at 0, 1, 2, 3, and 5 weeks of age (*solid black bars*). This figure also shows the 60-day survival rate of the mice pretreated with thymosin, or with a calf spleen or liver extract for the first 2 weeks of life and then challenged with the virus at 2 weeks (*striped bars*). As no significant difference in survival was observed between animals in the groups treated with either spleen or liver fractions, these groups are considered, for statistical analysis, as a single group. The survival of 2-week-old mice pretreated with thymosin was significantly higher than that found among saline treated 2-week-old animals ($X^2 = 5.132$, $0.025 > p > 0.02$). However, the survival of the 2-week-old thymosin-treated mice was not significantly different from that seen in untreated 3-week-old animals ($X^2 = 1.739$, $0.20 > p > 0.10$). As seen in Figures 1 and 2, the capacity of CBA/Wh mice to resist the progressive tumor growth caused by a murine sarcoma virus (Moloney) challenge is quite developed by 3 weeks of age. In contrast, treatment with liver or spleen extracts resulted in survival rates comparable to saline-treated 2-week-old animals ($X^2 = 0.149$, $p > 0.70$), but significantly lower than untreated 3-week-old animals ($X^2 = 9.304$, $0.005 >$

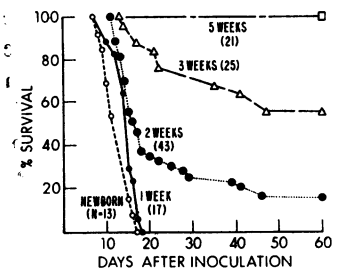

FIG. 1.—Influence of age of CBA/Wh mice on their resistance to progressive tumor growth. Age at time of inoculation as indicated; number of animals in each group in parentheses.

$p > 0.001$). These data indicate that pretreatment with thymosin accelerates the development of resistance to the lethal action of the virus. In contrast, treatment with other protein extracts prepared from calf tissue have no apparent effect on resistance of CBA/Wh mice to progressive tumor growth.

Discussion. Evidence that the thymus is involved in the maturation of resistance to the lethal effects of the Moloney virus-induced sarcoma has been indicated previously by the observation that thymectomy at 3 days of age significantly increases the incidence of progressive tumor growth in adult mice.[22] Restoration of resistance in neonatally thymectomized mice to the polyoma virus[23] and to syngeneic transplanted tumors[24] by the implantation of thymus grafts within cell-impermeable Millipore diffusion chambers provided indirect evidence for a significant humoral role for the thymus in the development of tumor immunity. Direct evidence has now been obtained in support of this hypothesis through the use of a cell-free thymic preparation, thymosin.

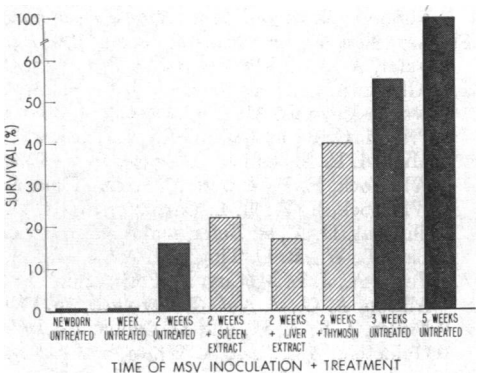

FIG. 2.—Development of resistance to progressive tumor growth in CBA/Wh mice inoculated with murine sarcoma virus (Moloney) at the times indicated. The groups consist of untreated controls or of mice treated with either thymosin or a calf spleen or calf liver fraction prior to viral inoculation. Schedule of treatment is in the text (*Materials and Methods*). The data depicted are the number of mice in each group (numbers in Table 1) that had survived 60 days after inoculation with th murine sarcoma virus (Moloney).

In view of the recent evidence that tumor regression is caused by cell-mediated reactivity against tumor-specific antigens and that thymosin enhances the development of delayed-type immune reactivity in neonatally thymectomized mice, it is significant that thymosin administration has been demonstrated to accelerate the development of resistance against virus-induced tumor growth in intact mice. Experiments are presently in progress to examine whether thymosin can similarly prevent progressive viral induced tumor growth in mice made immunologically deficient by irradiation, lymphotoxic drugs, or neonatal thymectomy. The present data suggest that the endocrine contribution of the thymus is a factor in the development of resistance to progressive tumor growth, and that the administration of thymosin to newborn mice can accelerate the development of normal host cell-mediated immunological competence.

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[20] The CBA mice of our colony have, for reasons described elsewhere[25] recently been designated as CBA/W to distinguish them from the CBA/J line of the Jackson Laboratories, Bar Harbor, Maine. It has been brought to our attention (Staats, J., *Cancer Res.*, **28**, 391 (1968)) that the designation W has been assigned previously to the Instytut Onkologii in Warsaw, Poland. Hence, we shall now refer to our CBA line as CBA/Wh.

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