Effects of Antithymus Serum and Antilymphocyte Serum on the Incidence of Lymphoid Leukemia* (34003)

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Importance of the thymus in determining susceptibility to lymphoid leukemia in mice and rats has been well established in a variety of ways (1) since McEndy *et al.* demonstrated that thymectomy reduced the incidence of lymphoid leukemia in AKR mice in 1944 (2). However, the mechanisms by which the thymus influences the development of lymphoid leukemia remain to be clarified (1). C3H mice injected with passaged Gross leukemia virus showed depressed immune responses and Ce/J skin grafts were not rejected by these mice suggesting a significance of depressed immunologic reactions in determining susceptibility to lymphoid leukemia (3, 4).

We have previously shown that antithymus serum has a greater immunosuppressive effect than antilymphocyte serum in rats and mice (5–9). Our recent study has indicated that antirat-thymus serum, but not antirat-lymphocyte serum, can produce lymphopenia in mice crossing species barriers and this may be mediated by antibody against a thymus-specific antigen common to the two species since the former can suppress thymidine uptake by mice thymocytes cultured *in vitro* (8).

The present study compared effects of antisera prepared against rat or mouse thymus or lymph node cells on the spontaneous incidence of lymphoid leukemia in AKR mice. The mean survival age of AKR mice treated with antirat-thymus serum was as short as that of mice treated with either antimouse-thymus serum or antimouse-lymphocyte serum. Treatment of AKR mice with either normal rabbit serum or antirat-lymphocyte serum which has no lymphopenic or immunosuppressive effects in the mice resulted in delay of death due to lymphoid leukemia.

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Materials and Methods. Inbred female AKR mice were obtained from West Seneca Laboratory, West Seneca, N. Y., at the age of 4 weeks. Three mice of the same experimental group were caged in each mouse pen with Purina laboratory chow and tap water *ad libitum*. Thymectomy was performed under sterile technique using vaccum suction (10). Antirat-thymus serum (ARTS) and antirat-lymphocyte serum (ARLS) were produced in rabbits using homogenates of thymus glands or mesenteric lymph nodes, respectively, obtained from 6 to 8 week old Osborne-Mendel strain rats as described previously (5). Antimouse-thymus serum (AMTS) was produced in rabbits by 5 weekly immunizations with a mixture (2.5 ml) of 20% saline suspension (by volume) of 6–8-week-old AKR thymus glands homogenized in a glass tube with a Teflon pestle and an equal volume of Freund's complete adjuvant. Antimouse-lymphocyte serum (AMLS) was prepared similarly using homogenates of mesenteric lymph nodes. One week after the last injection the rabbits were bled weekly for 3–4 months with a booster injection at monthly intervals. All the antisera were heated at 56° for 30 min, pooled, and absorbed with ½ vol of packed AKR mouse red cells.

Mice were divided into seven groups. Mice of Group I were left untreated. Group II mice underwent thymectomy at the age of 4 weeks. Completeness of thymectomy was confirmed at the time of death or sacrifice. Those mice with obvious residual thymus or lymphomatous masses in the anterior mediastinum suggestive of incomplete thymectomy were excluded from this study. Mice of Groups III, IV, V, VI, and VII were injected intraperitoneally with ARTS, ARLS, AMTS, AMLS, or control normal rabbit serum (NR S), respectively, in doses of 0.5 ml 3 times a week for 2 months between the age of 5 and 7 months.

TABLE I. Titers of Agglutinins and Cytotoxic Antibodies of Antirat-Thymus Serum (ARTS), Antirat-Lymphocyte Serum (ARLS), Antimouse-Thymus Serum (AMTS), Antimouse-Lymphocyte Serum (AMLS) and Normal Rabbit Serum (NRS) against AKR Mice Thymocytes, Lymphocytes, and Thymic Leukemia Cells.

	Agglutinin			Cytotoxicity antibody	
Rabbit serum	Lymphocytes	Thymocytes	Leukemic cells	Lymphocytes	Thymocytes
ARTS	1:64	1:64	1:64	1:128	1:128
ARLS	1:32	1:64	1:16	1:128	1:128
AMTS	1:64	1:64	1:64	1:256	1:512
AMLS	1:64	1:64	1:64	1:512	1:256
NRS	0	0	0	0	0

The presence or absence of lymphoid leukemia was confirmed by histological examinations under light microscopy of the thymus, spleen, liver, kidney, and mesenteric lymph nodes stained with hematoxylin and eosin. Mice dying prior to 9 months of age without demonstrable evidences of lymphoid leukemia were discarded from this study because they could have developed leukemia had they lived longer. Mice dying without demonstrable leukemia at or later than 11 months of age, on the other hand, were included in the study since more than 95% of untreated mice die of lymphoid leukemia on or before the age of 11 months and absence of histologically demonstrable leukemia in these mice is strongly indicative of a significant delay in leukemia development.

In vitro effects of ARTS, ARLS, AMTS, AMLS, and NRS were studied by agglutination and cytotoxicity tests using AKR mice thymocytes, lymph node lymphocytes, and leukemic cells of lymphomatous thymus with the method described previously (7) except that fresh guinea pig serum was absorbed with mouse spleen cells before it was used as a source of complement. *In vivo* effects of these antisera were assayed by their lymphopenic effects at 24 hr after intraperitoneal injection of 0.5 ml of the respective antiserum expressed as percentages of the initial peripheral lymphocyte counts. Immunosuppressive effects of AMTS and AMLS as determined by their abilities to prolong survival of skin allografts have been described elsewhere (8, 9). In order to assess immunosuppressive effects of ARTS and ARLS in mice, a full thickness graft of circular skin 14 mm in diameter was exchanged between 6 to 8-week-old female AKR ($H\text{-}2^k$) and CBA ($H\text{-}2^k$) or C57B1/6 ($H\text{-}2^b$) with the method described previously (5). Each mouse received 0.5 ml of either ARTS or ARLS or normal rabbit serum as control 3 times a week beginning on the day of grafting.

Results. Table I shows titers of agglutinating and cytotoxic antibodies of the four antisera and control rabbit serum. The lymphocyte counts at 24 hr after intraperitoneal injections of the antisera are expressed as mean percentages of the initial peripheral lymphocyte counts (Table II). It is to be

TABLE II. Peripheral Lymphocyte Counts 24 hr after Injection of Antisera Expressed as Percentages of Initial Lymphocyte Counts.

	Rabbit serum				
	ARTS	ARLS	AMTS	AMLS	NRS
Mean lymphocyte counts (% of initial counts)	20.0 ± 12.0[a]	97.4 ± 51.0	42.8 ± 17.8	44.2 ± 12.0	120.2 ± 63.6
	$0.001 < p < 0.01$[b]		$0.01 < p < 0.02$	$0.01 < p < 0.02$	

[a] Mean and SD of 7 animals for each group.

[b] Compared with NRS.

TABLE III. Immunosuppressive Effects of Anti-rat-Thymus Serum (ARTS) and Antirat-Lymphocyte Serum (ARLS) in Mice.

Antisera	Median survival time (days)	
	AKR-CBA	AKR-C57Bl/6
ARTS	>30 (11)[a]	16 (8)[a]
ARLS	16 (12)	9 (8)
Normal rabbit serum	13 (16)	9 (16)

[a] Number of animals.

noted that ARTS but not ARLS produced lymphopenia in mice. Table III shows immunosuppressive effects of ARTS and ARLS in mice. Immunosuppressive effects of ARTS can cross species barriers prolonging skin graft survival in mice whereas ARLS shows no such effects. The mean survival age for each of 7 groups of AKR mice is shown in Table IV. All the mice of Groups I and III through VII died at or before the age 16 months while only one of 29 successfully thymectomized mice of Group II died during the same period. Therefore, the remaining 28 thymectomized mice were sacrificed at that point. None of the 28 mice showed histologically demonstrable evidences of lymphoid leukemia. The treatment with NRS or ARLS seems to have delayed the mean age of mice dying from lymphoid leukemia (Fig. 1). Although the mean survival age for the group VII was significantly greater than that of the untreated group I mice, difference in the mean survival age between the group I and IV was not significant probably because of smaller numbers of animals ($0.05<p<0.1$).

Discussion. Whole-body irradiation induces lymphoid leukemia in mice, and shielding of part of the body or the injection of normal bone marrow or spleen cells after irradiation reduces the incidence of leukemia (11). This suggests that a breakdown of immune mechanisms by irradiation may be an important factor for induction of leukemia.

A significantly greater mean survival age in the group of mice treated with normal rabbit serum than that of the untreated mice and an apparent delay in leukemia death in the mice treated with NRS and ARLS may be due to nonspecific stimulation of immune

TABLE IV. Comparison of Mean Survival Ages between Groups.

Group:	I	II	III	IV	V	VI	VII
Treatment:	None	Thymectomy	ARTS	ARLS	AMTS	AMLS	NRS
No. of animals	50	29	16	18	22	20	42
Leukemia death	50	1	16	15	22	20	32
Nonleukemia death	0	0	0	3	0	0	10
Mean survival age (months)	8.2 ± 2.1[a]	—	8.3 ± 2.6	9.7 ± 3.4	8.9 ± 2.8	8.2 ± 2.6	10.0 ± 2.8
	$0.001<p<0.01$[b]		$0.02<p<0.05$		$0.1<p<0.2$	$0.02<p<0.05$	

[a] Standard deviation.
[b] Compared with Group VII.

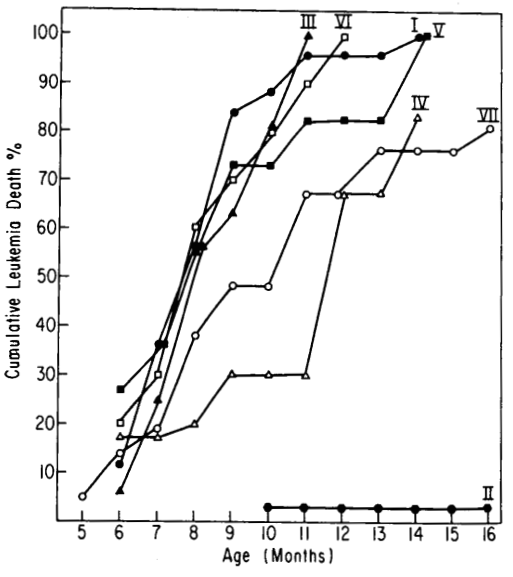

FIG. 1. Cumulative leukemia death in the seven groups of AKR mice.

responses by these sera. Injection of normal rabbit serum has been shown to increase thymidine incorporation by thymocytes (7).

As shown in Table III, ARTS demonstrated definite immunosuppressive effects in mice although it was prepared using rat thymus. The ARLS, on the other hand, showed no immunosuppressive effects in mice. This is consistent with our previous observations that antithymus serum has a greater immunosuppressive effect than antilymphocyte serum (5–9) suggesting qualitative or quantitative differences in their antibody activities (12). Lymphopenic effect of ARTS in mice crossing species barriers has been shown previously (8). Failure of ARLS to produce lymphopenia in the mice despite presence of agglutinating and cytotoxic antibodies in comparable titers may be another example for lack of correlation between *in vitro* tests and *in vivo* effects of antilymphocyte serum (13). Since ARTS as well as AMTS and AMLS (8, 9) prolonged skin graft survival in the mice it is possible that their immunosuppressive effects canceled out their nonspecific stimulatory effects on immune responses as foreign rabbit sera. It may be necessary to make mice tolerant to rabbit serum in order to evaluate pure immunosuppressive effects of these antisera.

Unlike lymphocytic choriomeningitis in mice which has been shown to be suppressed by antithymus serum (14) AMTS, AMLS, and ARTS seem to have no suppressive effects on the spontaneous incidence of lymphoid leukemia in AKR mice when they are given betewen 5 and 7 months of age. Effects of antithymus serum in adult animals can be equated to effects of neonatal thymectomy in its immunosuppressive actions (5–7). In contrast, the preventive effects of thymectomy on the spontaneous incidence of lymphoid leukemia in AKR mice are probably dependent more on maintenance of immune tolerance to the Gross virus (1, 15) than on immunosuppressive actions. Although thymectomy seems to be the most effective means to prevent the development of lymphoid leukemia in AKR mice, stimulation rather than suppression of immune responses appears to favor reduction in the incidence of spontaneous leukemia in nonthymectomized AKR mice.

Summary. Effects of antithymus serum and antilymphocyte serum on the spontaneous incidence of lymphoid leukemia in AKR mice were studied. Treatment of AKR mice with normal rabbit serum or antirat-lymphocyte serum which has no lymphopenic or immunosuppressive effects in the mice seems to delay death of the mice due to spontaneous lymphoid leukemia. Despite or, perhaps, because of their lymphopenic and immunosuppressive effects in the mice antirat-thymus serum, antimouse-thymus serum and antimouse-lymphocyte serum failed to alter the incidence of lymphoid leukmeia. Prolonged mean survival ages in the mice treated with either normal rabbit serum or antirat-lymphocyte serum might be attributed to their nonspecific stimulatory effects on immune responses.

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Effect of β-3-Thienylalanine on Antibody Synthesis III. Inhibition of RNA and Protein Synthesis in Immunized Rat Spleen Cells* (34004)

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Previous work from our laboratory (1–3) has indicated that β-3-thienylalanine (β-3-TA), an analog of the essential amino acid phenylalanine, when administered to rats by stomach tube induces marked depression of the production of specific antibody to sheep erythrocytes. This depression is seen when the analog is administered during the induction period and is not noted if β-3-TA is given beginning on the third day after antigen injection. The results of our previous experiments were interpreted as meaning that β-3-TA, like other compounds (4), has an effect on some events occurring during the induction period and will not act on antibody synthesis *per se* when given during the production phase. The purpose of the work reported here was to investigate further the mechanism by which B-3-TA depresses antibody production and to identify the step or steps which are affected by this analog.

This investigation has pointed out that one of the inhibitory effects is at the level of the synthesis of ribonucleic acid.

Materials and Methods. Sprague-Dawley, young adult, male, albino rats were used in all experiments. They were fed the diet described by Hruban and Wissler (5), prepared without addition of water, and with phenylalanin omitted when B-3-TA was administered. β-3-TA (Nutritional Biochemicals) was suspended in normal saline and heated to the boiling point until dissolved. The solubility of this compound in saline is poor, but by using this method, up to 70 mg/ml can be dissolved, being careful to restore the solution to the original volume after boiling. The preparation was always made up fresh as β-3-TA is rapidly decomposed in solution. Rats were immunized with 1 ml of 2% sheep erythrocytes intravenously on Day 0 and serial bleedings were done from the tail vein.

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