Feedback Mechanisms of Thymic Lymphocyte Production.* (32384)

HIROSHI NAGAYA AND HERBERT O. SIEKER
Department of Medicine, Duke University Medical Center, Durham, N. C.

We have previously shown that antithymus serum has more sustained and profound lymphopenic and immunosuppressive effects than antilymphocyte serum in rats although both antiserums had similar agglutinin titers against rat lymphocytes and thymocytes *in vitro*(1,2). The peripheral lymphocyte counts of rats receiving daily injection of antilymphocyte serum characteristically fluctuated at 5-day intervals(3) while antithymus serum produced sustained lymphopenia with little fluctuations(2). This suggested that there might be differences in the mechanisms of action between these antisera on the lymphoid tissues, especially on the thymus since only rats injected with antithymus serum showed marked lymphocyte depletion in the thymus (2).

In the present study we have investigated the effect of these antisera on uptake of tritiated thymidine or uridine by thymocytes attempting to correlate the thymic lymphocyte production with the changes in the peripheral lymphocyte counts.

Materials and methods. Antithymus serum (ARTS) was produced in albino rabbits (3 to 4 kg) by 5 weekly immunizations with a mixture of 20% saline suspension of homogenized thymus obtained from 6 to 8 week old Osborne-Mendel strain rats (not inbred, but kept in a closed colony for 38 to 39 generations) and an equal volume of Freund's complete adjuvant as described previously (1). Antilymphocyte serum (ARLS) was prepared similarly using homogenates of mesenteric lymph nodes. All the sera were heated at 56° for 30 minutes, pooled and stored at —20°C until used.

In vitro effects of these antisera were studied by agglutination tests and cytotoxicity tests using thymocytes and lymph node cells. Agglutination tests were performed with a slight modification of the method described previously(3). To 0.1 ml of the undiluted antisera and their serial 2-fold dilutions with saline, 0.05 ml of the cell suspension in a concentration of 30,000 to 50,000 cells per cubic millimeter were added and read microscopically after standing at 4°C overnight. For cytotoxicity tests, freshly removed thymus or mesenteric lymph nodes were teased in TC medium 199 (Difco). The cell concentrations were adjusted to 7 to 8 $\times$ 10^6 per milliliter. Five-hundredths milliliter of each cell suspension was added to a well containing 0.05 ml of the antiserum to be tested and 0.015 ml of fresh guinea pig serum as complement source. The well was incubated at 37° for 45 minutes and 0.04 ml of 0.5% trypan blue in saline was added to determine percentage of dead cells. The titers of the cytotoxic antibody were expressed by reciprocals of the maximum 2-fold dilutions of the serum with TC medium 199 giving more than 10% dead cells.

Three groups of 6-week-old male rats of Osborne-Mendel strain (100 to 120 g) received daily intraperitoneal injection of 1 ml of ARTS, ARLS or normal rabbit serum, respectively. The lymphocyte count was made daily on tail vein blood in each animal in order to confirm characteristic sequential fluctuations observed in rats receiving these antisera daily as described previously(2,3). Three animals from each group were sacrificed on Day 3, 5 and 9 after having demonstrated characteristic changes in the peripheral lymphocyte counts up to the time of sacrifice. The thymus gland from each rat was removed aseptically and teased in TC medium 199 (Difco) to obtain thymocyte suspensions. The cells were washed once in the same medium and suspended in a concentration of 10 to 12 $\times$ 10^6 cells per milliliter in a mixture of 80% Eagle's minimal essential medium, 20% fetal calf serum, 1% L-glutamine and 100 units of penicillin per milliliter of the medium.

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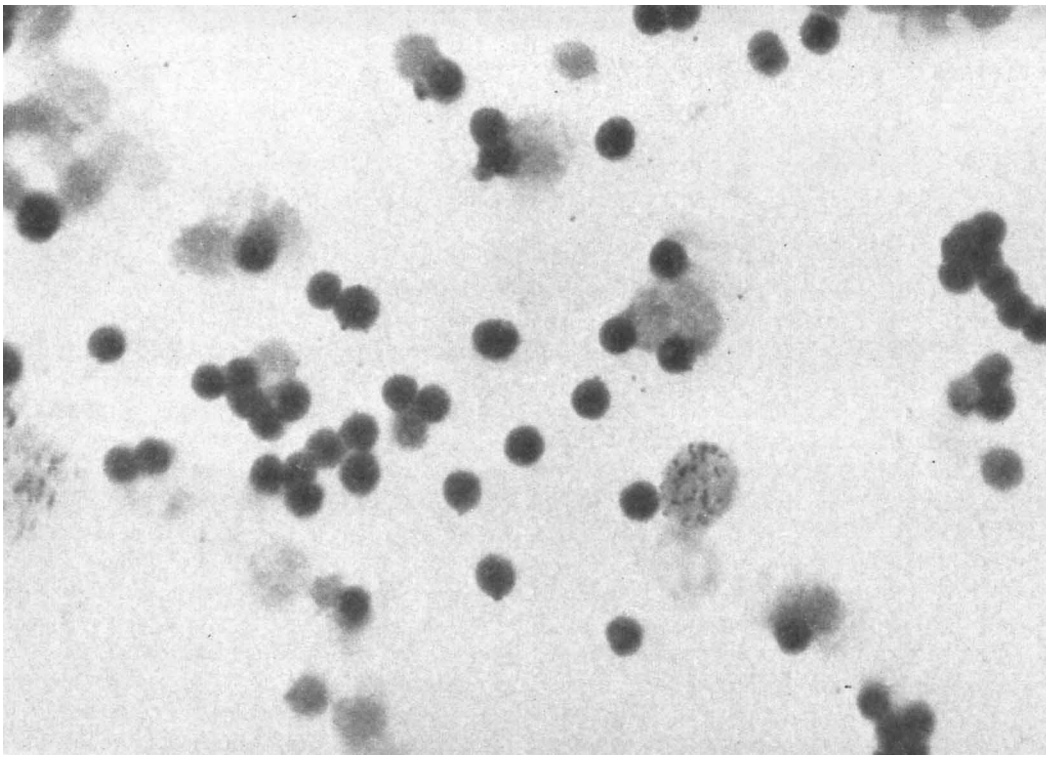

FIG. 1. Radioautograph of thymocytes from a rat which had received daily injection of antithymus serum for 3 days. The cells were incubated 1 hr with 2 μc of thymidine-H^3 per milliliter and the cell smears were exposed for 2 days. Note few cells with grains (× 540).

Thymidine-H^3 (2μc/ml, specific activity 6.0 c/mM)† or uridine-5-H^3 (5μc/ml, specific activity 8.0 c/mM)† was added to each of two thymocyte suspensions prepared from each animal and incubated at 37°C for one hour. At the end of one hour, the cells were washed and smeared on gelatin-coated slides and fixed in methanol. The slides were then dipped in Kodak Nuclear Track emulsion type NTB_2 and exposed for 2 to 7 days. The cells were then stained with May-Gruenwald-Giemsa (Fig. 1 and 2). A cell was defined as labeled when covered by 5 or more grains or if the number of overlying grains was at least 5 times the background grain counts noted in non-cellular zones. At least 1,000 cells were counted in each of 3 randomly selected fields for each animal and the mean value of 3 animals at each day of sacrifice was calculated for each of the 3 groups.

Results. The titers of agglutinin and cytotoxic antibody of ARTS and ARLS are shown in Table I. The titers of lymphocyte agglutinin and cytotoxic antibody were equal in both antisera and that of thymocyte agglutinin and cytotoxic antibody differed only by one dilution.

TABLE I. Titers of Agglutinins and Cytotoxic Antibody of Antithymus Serum (ARTS) and Antilymphocyte Serum (ARLS) Against Rat Lymphocytes and Thymocytes.

	Agglutinin titers		Cytotoxic antibody titers	
Rabbit serum	Lympho-cyte	Thymo-cyte	Lympho-cyte	Thymo-cyte
ARTS	1:128	1:128	1:64	1:256
ARLS	1:128	1:64	1:64	1:128
Normal	0	0	0	0

As described previously(2,3), the lymphocyte count of the rats receiving daily injection of ARLS showed the lowest count around the third day followed by the first rebound rise around the 5th or 6th day. The count fell again to a similar or lesser degree

† Schwarz Bio-Research, Inc., Orangeburg, N. Y.

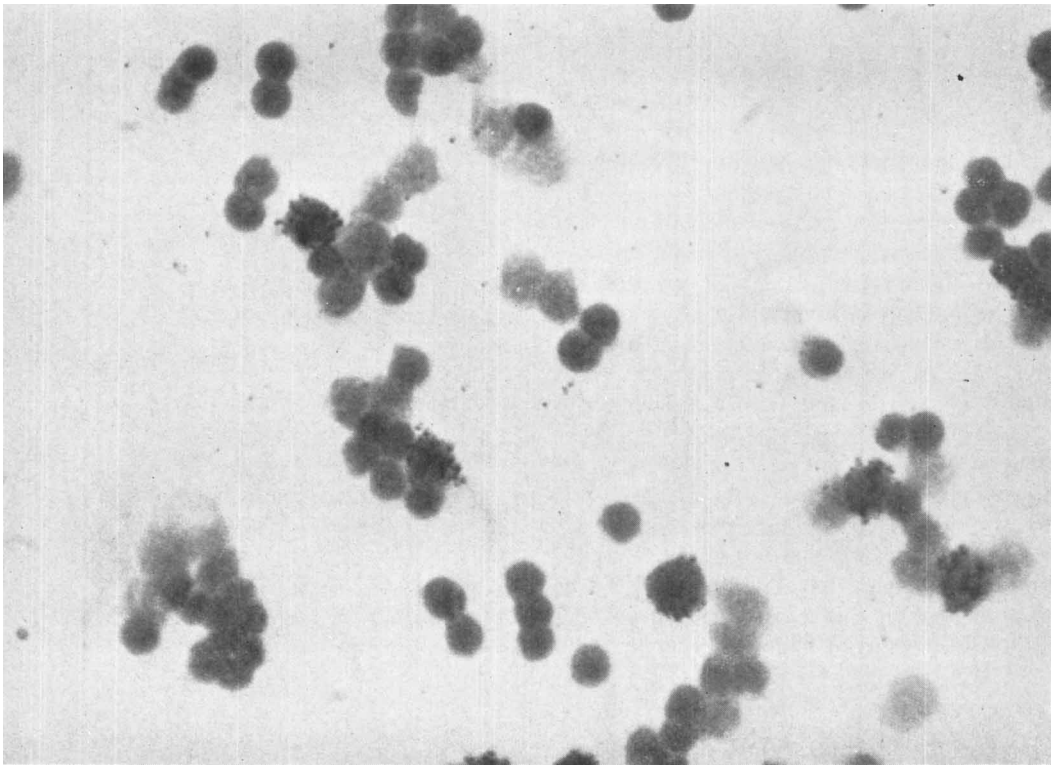

FIG. 2. Radioautograph of thymocytes from a rat which had received daily injection of antilymphocyte serum for 3 days. The thymocytes were incubated 1 hr with 2 μc of thymidine-H^3 per milliliter and the cell smears were exposed for 2 days. Note many cells with grains ($\times$ 540).

TABLE II. Numbers of Thymocytes Labeled with Thymidine-H^3 *in vitro* per 1,000 Cells Obtained from Rats Injected with Antithymus Serum (ARTS), Antilymphocyte Serum (ARLS) or Normal Rabbit Serum (NRS) Daily.

Injected serum	Days of sacrifice 3	5	9
ARTS	33.0 $\pm$ 6.5*	10.3 $\pm$ 3.5	105.7 $\pm$ 3.6
ARLS	89.3 $\pm$ 3.3	38.3 $\pm$ 3.0	162.7 $\pm$ 8.4
NRS	70.3 $\pm$ 7.9	67.0 $\pm$ 2.4	158.3 $\pm$20.0

* Standard deviation.

TABLE III. Numbers of Thymocytes Labeled with Uridine-5-H^3 *in vitro* per 1,000 Cells Obtained from Rats Injected with Antithymus Serum (ARTS), Antilymphocyte Serum (ARLS) or Normal Rabbit Serum (NRS) Daily.

Injected serum	Days of sacrifice 3	5	9
ARTS	40.0 $\pm$ 3.6*	30.2 $\pm$ 3.5	34.0 $\pm$ 2.2
ARLS	40.3 $\pm$ 5.2	17.9 $\pm$ 1.6	20.3 $\pm$ 6.2
NRS	20.0 $\pm$ 1.0	9.6 $\pm$ 2.2	8.7 $\pm$ 1.4

* Standard deviation.

on the 7th or 8th day returning toward the original value on the 9th or 10th day. In contrast, the count of the rats receiving ARTS showed only minimal rebound rise on the 5th or 6th day although the lymphopenia of a comparable degree was observed around the 3rd or 4th day. The lymphocyte count fell gradually following the minimal rebound rise with little upward deflection thereafter.

The results of incorporation of tritiated thymidine or uridine by thymocytes *in vitro* are shown in Tables II and III. It can be seen that thymidine incorporation of thymocytes obtained from rats receiving ARTS was significantly lower than that in the two other groups. The number of thymocytes labeled with thymidine-H^3 *in vitro* per 1,000 cells obtained from rats sacrificed prior to any serum injection was 47.0 $\pm$ 1.5. Therefore, the thymidine uptake seemed to be increased even in the animals receiving normal rabbit serum possibly because of antigenic stimu-

lation by the injected foreign serum. The animals receiving ARLS, on the other hand, had a significantly higher number of labeled cells than that observed in the control group on Day 3 ($P<0.05$) when the peripheral lymphocyte count was low, but the thymidine uptake in thymus was significantly less than that of the control group ($P<0.01$) on Day 5 when the blood lymphocyte count returned toward the original value. In contrast, the uridine uptake of thymocytes obtained from rats receiving ARTS was significantly higher than that of the control group on Day 3, 5 and 9 ($P<0.01$) and was also higher than that of the group receiving ARLS on Day 5 and 9 ($P<0.05$). Since 11.7 $\pm$ 0.7 per 1,000 thymocytes obtained from rats sacrificed prior to any serum injection were labeled with uridine, it appears that injection of foreign protein alone may possibly increase the uptake as seen in the control group on Day 3 although it returned to the preinjection value on Day 5 and 9.

Discussion. Although there was no apparent morphological difference between cells labeled with thymidine and uridine on smears, our preliminary study using radioautograph of the paraffin sections of the thymus obtained from rats injected with tritiated uridine intravenously indicates that uridine labels appear to be more in the reticulo-epithelial cells and concentrated in the medulla of the thymus. If we assume that thymidine uptake by thymocyte reflects lymphocyte production in thymus, and uridine uptake indicates RNA metabolic activity in thymus which may reflect production of a thymic humoral factor, since thymus does not produce antibody protein, the data described can be interpreted in the following way. In animals treated with ARLS, production of a thymic humoral factor in the thymus increases in response to lymphocyte destruction in the blood as evidenced by the sustained increase in uridine uptake over the control group (Fig. 3). Consequently, the blood level of a thymic humoral factor will be raised which in turn stimulates the thymic lymphocyte production as indicated by the increased thymidine uptake on Day 3. However, the thymidine uptake on Day 5 is decreased when the blood lymphocyte count returns toward normal. The periodic fluctuations of the lymphocyte count at 5-day intervals may be explained by the findings that the thymus is the major source of small lym-

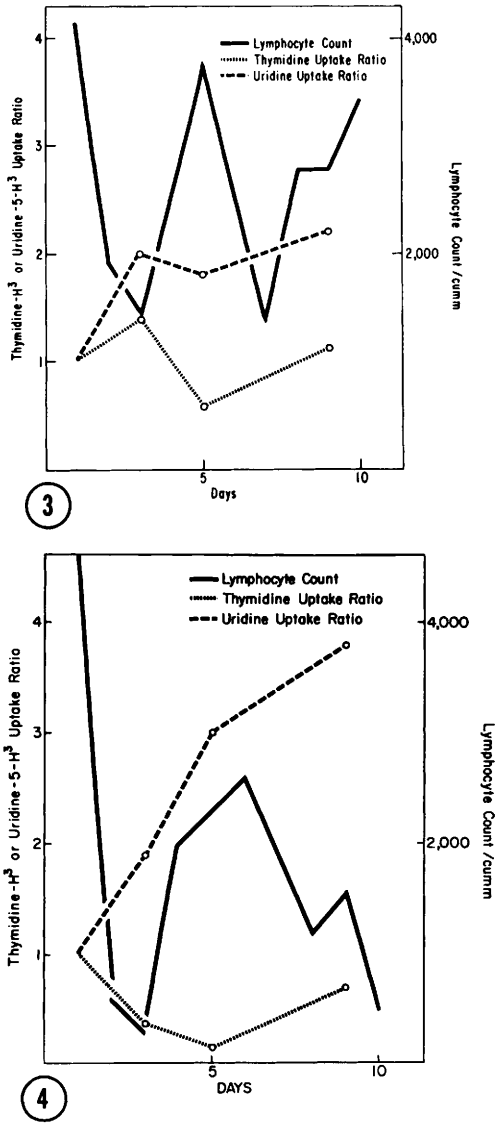

FIG. 3. Effect of daily injection of antilymphocyte serum on peripheral lymphocyte count and uptake of thymidine-H^3 or uridine-5-H^3 by thymocytes *in vitro*. The uptake was expressed by a ratio of numbers of labeled cells between the group receiving antilymphocyte serum and the control group.

FIG. 4. Effect of daily injection of antithymus serum on peripheral lymphocyte count and uptake of thymidine-H^3 or uridine-5-H^3 by thymocytes *in vitro*. The uptake was expressed by a ratio of numbers of labeled cells between the group receiving antithymus serum and the control group.

phocytes of short life span and their generation or replacement time has been shown to be 3 to 5 days(4,5). In fact, if rats were thymectomized prior to daily injection of ARLS, no periodic rises in the lymphocyte count were observed. Therefore, it is possible that the thymus releases small lymphocytes into the circulation when lymphopenia develops, although the majority of these cells may die within the organ if peripheral lymphoid tissues are intact(6).

In animals treated with ARTS the uridine uptake of thymocytes was increased and the increase continued over the 9 days as indicated by the uptake ratio (Fig. 4). However, the thymidine uptake remained lower than that of the control group and the lymphocyte count in the blood also stayed depressed despite an increased production of a thymic humoral factor in thymus as suggested by the uridine uptake. It is possible that ARTS contains antibody against a thymic humoral factor and continuously inactivates it in the circulation. Because of decreased level of a thymic humoral factor in the blood, lymphocyte production in thymus remains depressed despite compensatory increase in the production of a thymic humoral factor in thymus glands. The possibility that in rats receiving ARTS depletion of thymic small lymphocytes was mediated by a decreased level of a thymic humoral factor in the blood is further supported by the findings that no rabbit serum was detected in the imprints of the thymus of these animals by indirect immunofluorescent techniques and there were no signs of inflammatory reaction in the thymus histologically to account for the depletion of small lymphocytes by direct toxic effect of the antiserum.

Summary. Thymidine-H^3 uptake of thymocytes from rats injected with antilymphocyte serum was increased when the blood lymphocyte count was low whereas it was decreased when the count returned to normal. Thymocytes from rats injected with antithymus serum showed increased RNA metabolism despite lowered thymidine-H^3 uptake and sustained lymphopenia. The increased RNA metabolism may indicate a compensatory increase in the production of a thymic humoral factor which was inactivated by antithymus serum in the blood resulting in sustained lymphopenia.

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Demonstration of an Early Host-Graft Incompatibility Reaction In Radiation Chimeras with the Jerne Plaque Technique.* (32385)

N. GENGOZIAN, C. F. GOTTLIEB, AND E. M. ALLEN
Medical Division, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tenn.

It is well documented(1,2) that mice can be protected from the effects of lethal total-body irradiation by injection of isogeneic, allogeneic, and xenogeneic bone marrow shortly after exposure. A variety of techniques has been used to demonstrate that this protection is due to transplantation of a complete donor hematopoietic system into the radiated host. With foreign-marrow treatment, a major

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