STUDY ON CELLULAR EVENTS IN POST-THYMECTOMY AUTOIMMUNE OOPHORITIS IN MICE

II. Requirement of Lyt-1 Cells in Normal Female Mice For the Prevention of Oophoritis*

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Various organ-specific autoimmune diseases can be readily induced in some strains of mice by thymectomy during the critical neonatal period (NTx),[1] on day 2–4 after birth, without any exogenous sensitization with autoantigen (1–4). It is assumed in this model that NTx may eliminate T cells that would otherwise ontogenically peripherize via the thymus after the day of thymectomy (Tx), and may consequently allow the residual population of T cells that have already peripherized before Tx to respond to self-antigen(s) and eventually to cause autoimmune diseases in adulthood. In other words, NTx may deplete a subpopulation of T cells that in the normal state should suppress or regulate self-reactive clones. If this assumption is correct, then it should be possible to prevent the development of autoimmune disease by reconstituting NTx mice with the appropriate lymphoid cells. In fact, it was demonstrated that autoimmune diseases induced by NTx could be prevented by inoculation of splenic T cells or thymocytes from normal syngeneic adult mice (4–6).

In this study we have attempted to characterize the regulatory T cell population that is capable of preventing autoimmune oophoritis by cell surface antigens, particularly Lyt antigens, and other immunobiological features. The cells responsible for preventing the development of oophoritis in the spleens were found to be Thy-1^+, Lyt-1^+,23^- cells, and were not eliminated by adult thymectomy (ATx). Thymocytes having such a preventive capacity were also found to be Lyt-1^+,23^-. Thus, the depletion of this Lyt-1 regulatory subpopulation by NTx may result in the development of oophoritis as well as other post-thymectomy autoimmune diseases.

Materials and Methods

Mice. A/J mice were kindly provided by Dr. K. Moriwaki, Institute for Genetics, Misima, Japan; and both B10.A(3R) and B10.A(5R) mice were kindly provided by Dr. M. Taniguchi,

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[1] *Abbreviations used in this paper:* ATS, antithymocyte serum; Lyt-1, Lyt-1^+,23^-; ATx, adult thymectomy; C, complement; CY, cyclophosphamide; Lyt-1,23, Lyt-1^+,23^+; Lyt-23, Lyt-1^-,23^+; NMS, normal mouse serum; NOx, neonatal oophorectomy; NTx, neonatal thymectomy, T_{DTH}, T cell mediating delayed-type hypersensitivity; T_H, helper T cell; T_S, suppressor T cell.

Chiba University School of Medicine, Chiba, Japan. All other mice used have been described elsewhere (7). The T cell surface phenotype of A/J is Thy-1.2, Lyt-1.2, 2.2, 3.2, TL-1,2,3, Qa-1^+, $Ia^{k/d}$, and $I\text{-}J^k$.

Antisera. Specificities and titers of antisera against Lyt, TL, Qa-1, and Ia^k antigens have been described elsewhere (7). For preparation of anti-$I\text{-}J^k$ serum, B10.A(3R) female mice were immunized several times with B10.A(5R) spleen cells and lymph nodes cells. The cytotoxic titer of the anti-$I\text{-}J^k$ antiserum was kindly assayed by Dr. M. Taniguchi by testing against T cell hybridoma lines expressing $I\text{-}J^k$ antigen (8). The antiserum killed 50% of target cells at a dilution of 1:20. The antiserum was used after being absorbed with thymocytes of B10.A(3R).

Complement (C) -dependent Cytotoxicity Assay. This assay, the trypan blue dye exclusion test, was carried out according to the method of Boyse et al. (9) using preselected rabbit serum for low toxicity and high C activity as C source. Bulk treatment of spleen cells or thymocytes with various alloantisera plus C were performed as described elsewhere (7). Anti-Tl-1,2,3 and anti-$I\text{-}J^k$ were used for bulk treatment at dilutions of 1:10 and 1:5, respectively.

T Cell Enrichment and Removal of Dead Cells and Erythrocytes (RBC). Spleen T cells were enriched by passage through a nylon-wool column (Wako Chemical, Ltd., Osaka, Japan) (10) or by "panning" on dishes coated with anti-mouse Ig (11). Goat anti-mouse Ig, purified by affinity column, was kindly provided by Dr. K. Okumura, Tokyo University School of Medicine, Tokyo, Japan. The proportion of Thy-1^+ cells obtained by either procedure was usually 85–90%. For the cytotoxicity test, the spleen cells thus processed were removed of dead cells and RBC by Ficoll-Isopaque centrifugation.

Thymectomy (Tx) and Oophorectomy (Ox). NTx was performed on day 3 (the day of birth was day 0) according to procedures described elsewhere (1). Generally, 90% of NTx A/J mice developed autoimmune oophoritis in adulthood (12). For the prevention experiments, spleen cells or thymocytes were obtained from normal adult female mice. The cells were injected intraperitoneally into NTx mice on day 10 (1 wk after NTx) unless otherwise specified. ATx was performed at 1 mo of age by the suction technique and 1 mo later ATx mice were used for experiments. Bilateral Ox was performed within 24 h after birth (NOx) or on day 7 (day-7-Ox) under a dissecting microscope by retroperitoneal incision.

Histological Examination and Indirect Immunofluorescence (IF) Technique. When mice were killed, sera were collected for the examination of autoantibodies by indirect IF (7). Ovaries and other organs were prepared for histological examination.

In Vivo Treatment with Cyclophosphamide (CY) or Antithymocyte Serum (ATS) and In Vitro Irradiation. CY (Shionogi Co. Ltd., Osaka, Japan), 200 mg/kg or 0.5 ml of ATS, prepared as described elsewhere (7), was injected intraperitoneally into normal adult female A/J mice 3 d before killing, and these mice were used for the prevention experiments. Spleen cells from normal adult female A/J mice were X irradiated in vitro just before use with a dose of 400 rad by a Toshiba X-ray source at a dose rate of 90 rad/min.

Results

Prevention of the Development of Oophoritis in NTx Mice by Normal Spleen Cells. During the course of our present experiments, the incidence of oophoritis in NTx A/J mice at 2 mo of age was 172/196 (90%). At various intervals after or before NTx, attempts were made to prevent the development of oophoritis by a single intraperitoneal injection of 2×10^7 spleen cells obtained from normal adult female A/J mice. As shown in Table I, the disease could be completely prevented if NTx mice were reconstituted within 2 wk after NTx or preinjected before NTx. However, the effect of this reconstitution decreased if the time of the treatment was delayed until 3 wk and was completely lost 4 wk after NTx. Based on these results, the treatments for disease prevention were carried out 1 wk after NTx in the following experiments.

Prevention of the Development of Oophoritis with Spleen Cells from Adult but not from Newborn or Infant Mice. Spleen cells (2×10^7) from young adult mice, older than 1 mo, were able to prevent the development of the oophoritis completely but those from newborn

TABLE I

*Normal Spleen Cells Can Prevent Oophoritis When Injected within 2 Wk after NTx**

2×10^7 spleen cells injected on	Number oophoritis/ total	Effect on prevention
Day 0	0/5	+
Day 10	0/10	+
Day 17	0/10	+
Day 24	2/10	±
Day 31	8/10	−
NTx control‡	17/20	

* The A/J female mice that were thymectomized on day 3 after birth (NTx) were reconstituted intraperitoneally with 2×10^7 spleen cells from adult female mice at various times after birth and killed at 2 mo of age for histological and serological examinations. Development of oophoritis was assessed by histology of ovaries, i.e., damage and loss of oocytes and corpora lutea accompanying infiltration of inflammatory cells (see ref. 7). Histological development of the oophoritis was generally accompanied by the appearance of circulating autoantibodies detected by IF. (Titer ≧ 1/10 dilution.)

‡ No spleen cells were injected into NTx A/J mice.

TABLE II

*Spleen Cells from Adult Mice, but not from Newborn or Infant, Can Prevent Oophoritis**

2×10^7 spleen cells obtained from the mice at the age of	Thy-1^+ cells in spleen‡	Number of oophoritis/total	Effect on prevention
7 d	<5%	8/9	−
14 d	<5%	5/10	±
21 d	13%	0/10	+
1 mo	23%	0/10	+
2 mo	28%	0/10	+
NTx control§		18/20	

* NTx A/J female mice were injected intraperitoneally 1 wk after NTx with 2×10^7 spleen cells from mice of various ages and killed at 2 mo of age for histological and serological examinations. See also footnote to Table I.

‡ Thy-1^+ cells were examined by cytotoxicity tests (mean percent of three experiments).

§ No spleen cells were injected into NTx A/J mice.

(7-d) or infant (14-d) mice were not (Table II). The proportion of Thy-1^+ cells in spleens was determined by cytotoxicity tests and was found to be <5% until 2 wk of age, gradually increasing to 30% in adults.

Reduced Capacity of Spleen Cells and Thymocytes from NOx Mice to Prevent Oophoritis. As shown in Table III, $>1 \times 10^7$ spleen cells were able to prevent the development of oophoritis completely. However, when the spleen cells were obtained from adult NOx mice, they were less competent, i.e., four times more spleen cells (4×10^7) were required for the prevention. This reduced preventive capacity did not seem to be due to hormonal effects upon lymphocytes after Ox, since spleen cells from the adult day-7-Ox mice had the preventive capacity equivalent to those from normal adult female mice.

1×10^7 thymocytes from normal female mice could prevent the development of

TABLE III

Reduced Capacity of Spleen Cells from NOx Mice to Prevent Oophoritis

Cell dose inoculated	Source of spleen cells used for prevention*		
	Incidence of Oophoritis (number of Oophoritis/ total number)		
	Normal mice	NOx mice	Day-7-Ox mice
1×10^6	6/6	—	—
5×10^6	5/10	6/7	—
1×10^7	0/20	5/10‡	0/10
2×10^7	0/10	3/10	—
4×10^7	0/10	0/10	—
NTx control§		18/22‖	

* Spleen cells were obtained from the adult female mice or mice that had received oophorectomy within 24 h after birth (NOx), or on day 7 after birth (day-7-Ox). Graded number of spleen cells was inoculated into NTx mice at 1 wk after NTx. These mice were killed at 2 mo of age for examination of the development of autoimmune disease. Occurrence of disease was assessed by histological examination.

‡ Significantly higher incidence compared with that in NTx mice inoculated with the same dose of spleen cells from normal or day-7-Ox mice. $P < 0.01$ by χ^2 test.

§ No spleen cells were injected into NTx A/J mice.

‖ Incidence of oophoritis in 196 NTx A/J female mice observed during the course of our experiments was 90%.

TABLE IV

Reduced Capacity of Thymocytes from NOx mice to Prevent Oophoritis

Cell dose inoculated	Source of thymocytes used for prevention*		
	Incidence of oophoritis (number oophoritis/ total number)		
	Normal mice	NOx mice	Day-7-Ox mice
5×10^6	5/9	—	—
1×10^7	1/10	8/10‡	1/8
2×10^7	1/10	6/10§	0/8
4×10^7	0/10	1/7	—
NTx control‖		16/18	

* Thymocytes were obtained from adult female mice or mice which had been oophorectomized within 24 h after birth (NOx) or on day 7 after birth (day-7-Ox). Graded numbers of thymocytes were inoculated into NTx mice 1 wk after NTx. These mice were killed at 2 mo of age for examination of the development of oophoritis.

‡ Significantly higher incidence of oophoritis compared with that in NTx mice inoculated with the same dose of thymocytes from normal or day-7-Ox mice. $P < 0.01$ by χ^2 test.

§ Significantly higher incidence: $P < 0.02$.

‖ No thymocytes were injected into NTx A/J mice.

oophoritis, as shown in Table IV. However, 4×10^7 thymocytes were required when they were obtained from NOx female mice. Thymocytes from day-7-Ox mice had almost the same preventive capacity as those from normal female mice.

Lyt-1 Spleen Cells and Lyt-1 Thymocytes Can Prevent Oophoritis. Cell surface antigens

TABLE V

Cell Surface Phenotype of Preventing Cells in Spleen: Requirement of Lyt-1$^+$,23$^-$, Qa-1$^-$, Ia$^-$ Cells for Prevention

Treatment of spleen cells*	Experiment Number																	Number oophoritis/ total
	1 (4)†	2 (4)	3 (4)	4 (3)	5 (4)	6 (5)	7 (3)	8 (5)	9 (4)	10 (4)	11 (4)	12 (4)	13 (4)	14 (4)	15 (5)	16 (4)	17 (4)	
NMS	−§	−	−		−	−	−	−	−									0/8
Anti-Thy-1.2											+	+	+	+	−	+	+	6/7
Anti-Lyt-1.2	+	−	+	−	−	+		+	+	+	+	+	+	+	+	+		12/15
Anti-Lyt-2.2										−	−		−	−		−	−	0/8
Anti-Lyt-3.2	−	−	−	−	−	−		−										0/7
Anti-Ia^k						−	−	−		−		−			−		−	0/7
Anti-Qa-1									−		−		−	−	−	−	−	0/7
NTx control‖	−	+	+	+	+	+	+	+	+	+								9/10

* Spleen cells (4×10^7) obtained from adult female A/J mice were treated with various alloantisera plus C and thereafter inoculated into NTx A/J mice at 1 wk after Tx. These recipient NTx mice were sacrificed at 2 mo of age for histological and serological examinations to see the development of oophoritis. See also Fig. 1.

‡ Number of one littermate indicated in parentheses. Each experiment was performed using one littermate of female NTx A/J mice.

§ −, Oophoritis in NTx A/J mice was prevented; +, oophoritis was not prevented.

‖ No spleen cells were injected into NTx A/J mice.

TABLE VI

Prevention with Nylon-Wool Nonadherent Spleen Cells: Requirement of Lyt-1$^+$, Qa-1$^-$, Ia$^-$, I-J$^-$ Cells for Prevention

Treatment of cells*	Experiment Number									Number of oophoritis/ total
	1 (5)‡	2 (3)	3 (3)	4 (4)	5 (5)	6 (4)	7 (3)	8 (4)	9 (4)	
NMS	−§						−		−	0/3
Anti-Lyt-1.2	+	−	+	+	+	+	+	+	+	8/9
Anti-Lyt-2.2	−				−	−	−	−		0/5
Anti-Qa-1 + anti-Lyt-2.2‖	−	−	−	−	−					0/5
Anti-Ia^k + anti-Lyt-2.2‖	−	−	−	−	+	−		−	−	1/8
Anti-I-J^k + anti-Lyt-2.2‖				−	−	−		−	−	0/5

* Cells were readjusted to 5×10^6 after treatment and then inoculated into NTx A/J mice.

‡ Number of one littermate indicated by parentheses. Each experiment was performed using one litter of female NTx A/J mice.

§ See footnote in Table V.

‖ Anti-Lyt-2.2 was mixed with anti-Qa-1, anti-Ia^k, or anti-I-J^k serum and used for the treatment of nylon wool nonadherent spleen cells obtained from normal adult female mice, e.g., Qa-1$^-$ Lyt-1$^+$ cells were prepared by simultaneous treatment with anti-Lyt-2.2 and anti-Qa-1 plus C.

of the preventing cells in spleen and thymus were studied (Table V). When spleen cells were removed of Thy-1$^+$ cells by anti-Thy-1 plus C, the preventive capacity was eliminated, whereas spleen cells removed of Ig$^+$ cells by anti-Ig and C was able to prevent the development of oophoritis. Nylon wool-nonadherent spleen cells (5×10^6) retained their preventive capacity (Table VI). All these results suggested that T cells were responsible for preventing oophoritis in NTx mice. These splenic T cells were further classified into subpopulations according to Lyt phenotypes. When the spleen cells treated with anti-Lyt-1 plus C were inoculated into NTx mice, oophoritis developed with similar incidence to control NTx mice (Table V). In contrast, Lyt-1

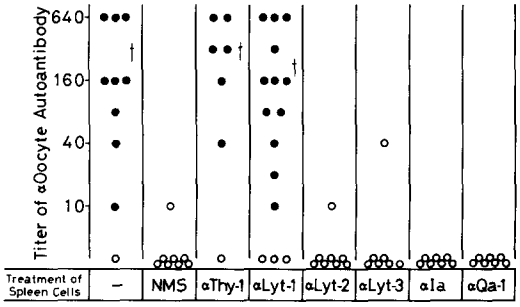

FIG. 1. Titer of autoantibody against oocytes in the sera of individual mice used in the experiment shown in Table V. Autoantibody was examined by indirect IF against cryostat sections of mouse ovary. Ooplasm and/or zona pellucidae were stained specifically with the sera taken from the mice with oophoritis (●), but were not stained with the sera taken from mice that did not develop oophoritis histologically (○). Vertical bars show the standard errors of the means. NTx control: no spleen cells were injected into NTx mice.

TABLE VII

Cell Surface Phenotype of Preventing Cells in the Thymus

Treatment of thymocytes	Number of cells treated*	Number of oophoritis/total number	Effect on prevention
NMS	4×10^7	0/10	+
NMS	5×10^6	6/10	–
Anti-Lyt-3.2	4×10^7	0/12	+
Anti-TL-1,2,3	4×10^7	2/10	+
NTx control‡	—	9/10	

* NTx mice were inoculated at 1 wk after Tx with A/J thymocytes which had been treated with NMS, anti-Lyt-3.2, or anti-TL-1,2,3 plus C. When thymocytes (4×10^7) were treated with Lyt-3.2 and C, $3–4 \times 10^6$ Lyt-1 thymocytes remained. When the same number of thymocytes was treated with anti-TL-1,2,3 plus C, $4–5 \times 10^6$ TL^- thymocytes remained. The cells thus treated were inoculated into NTx A/J mice.

‡ No thymocytes were injected into NTx A/J mice.

cells that remained after treatment with anti-Lyt-2 or anti-Lyt-3 plus C completely prevented the disease; furthermore, removal of Qa-1^+ or Ia^+ cells did not affect the preventive capacity. When nylon-wool nonadherent cells were treated with various antisera plus C and inoculated after the cell number was readjusted to 5×10^6, Lyt-1 cells were found to retain the preventing capacity. These Lyt-1 preventive cells also lacked Qa-1 and Ia antigens, including I-J antigen (Table VI).

Titers of circulating autoantibodies against oocytes in individual mice in Table V were examined by IF (Fig. 1). In accordance with the results of histological examination, Lyt-1^+,23^-, Ia^-, and Qa-1^- spleen cells clearly prevented the generation of anti-oocyte autoantibodies.

Thymocytes are generally considered to be classified among Lyt-1^+,23^- and Lyt-1^+,23^+ subpopulations (13, 14). When 4×10^7 thymocytes were treated with anti-Lyt-3 and C, the remaining $3–4 \times 10^6$ Lyt-1 thymocytes were able to prevent the development of oophoritis, as shown in Table VII. On the other hand, 5×10^6 untreated thymocytes were not able to prevent the disease, which suggests that the preventive capacity is present in the Lyt-1 thymocyte subpopulation. When 4×10^7 thymocytes were treated with anti-TL-1,2,3 and C, the remaining $4–5 \times 10^6$ TL^-

thymocytes could prevent the disease.

Sensitivity of Preventing Cells to Low-dose X Irradiation, CY, ATS Treatment, or ATx. When spleen cells (1×10^7) were irradiated in vitro with 400 rad X irradiation, the preventive capacity decreased significantly, i.e., 5 out of 10 NTx mice inoculated with irradiated spleen cells developed oophoritis. CY at a dose that depletes B cells (15) or ATS gave no effect on the preventing activity at all. Thymectomy of spleen cell donors at 4 wk of age (ATx) did not influence the preventive capacity of the spleen cells and it was found that oophoritis was prevented in all 10 NTx mice.

Decrease in Numbers of Lyt-1 Cells in the Spleens of NTx Mice. The number of spleen cells generally diminished soon after NTx but gradually increased to almost the same level as that of control, non-Tx mice. The proportion of Thy-1^+ cells in control A/J mouse spleens was usually 25–30% but decreased in NTx mice to 15–20% at 2 mo of age. The composition of Lyt subsets in T cell-enriched spleen cells was examined by cytotoxicity test. Lyt subsets among splenic T cells in normal adult A/J and NTx mice were as follows: non-Tx control, Lyt-1 45%, Lyt-1,23 50%, Lyt-23 negligible, if present, and Lyt^- 5%; NTx: Lyt-1 25%, Lyt-1,23 70%, Lyt-23 negligible, if present, and Lyt^- 5%. These results suggested that the decrease in numbers of Thy-1^+ cells in NTx mice was predominantly among those in the Lyt-1 subpopulation.

Discussion

Our present experiments elucidated some of the immunobiological characteristics of the preventing cells in spleen and thymus that can inhibit the development of oophoritis in NTx A/J mice. When the spleen cells or thymocytes were derived from NOx mice, four times as many cells were required for prevention, which suggested that the development of the preventing cells was hindered by NOx. Since Ox on day 7 after birth did not interfere with the preventive capacity, it is conceivable that normal female mice develop this population through recognition of ovarian autoantigen(s) during the fetal or neonatal period. Furthermore, these cells do not seem to need continuous replenishment from the thymus because ATx did not influence the capacity of spleen cells to prevent oophoritis development. In our previous report (7), a requirement of Lyt-1 effector cells similar to T cells mediating delayed-type hypersensitivity (T_{DTH}) for the destruction of ovaries was demonstrated in adoptive transfer experiments with newborn and athymic nude mice, although Lyt-1 helper T cells (T_H) for autoantibody formation may also play some role in the nude mouse transfer. The present experiments demonstrated that the preventing cells were able to suppress not only the development of T_H for autoantibody formation but also that of T_{DTH}-like effector cells (Fig. 1, Table V).

The population of lymphocytes having the potential to prevent the development of oophoritis was revealed to be T cells belonging to an Lyt-1 subset both in the thymus and the spleen. This result seems to correlate well with the finding that the number of Lyt-1 cells were substantially decreased in NTx mice. Although there is still some controversy concerning the ontogenic pathways of the different Lyt subsets, it is generally believed that the Lyt-1 subset in the thymus migrates to peripheral lymphoid organs and constitutes a part of the Lyt-1 population (13, 14, 16). Considering that Lyt-1 thymocytes and Lyt-1 spleen cells have almost the same potential for preventing the development of oophoritis, it is likely that the Lyt-1 preventing cells in the thymus migrate and constitute the Lyt-1 preventing cells in spleen and lymph nodes. The Lyt

phenotype of this preventing cell population is in agreement with the results by others on that suppressor T cells (T_S) for DTH that are Lyt-1 and also lack Ia antigen (17, 18). T_S with Lyt-23 phenotype was also reported in several systems (19, 20). Both these Lyt-1 and Lyt-23 T_S, however, were found to suppress the effector phase of T_H or T_{DTH} (17–20) and contrast with our Lyt-1 preventing cells, which seemed to suppress the development of effector cells but not to suppress the function of effector cells already developed, since preventive treatment upon adult NTx mice developing overt oophoritis was ineffective (Table I).

Recently, a multistage suppression or regulatory circuit hypothesis was proposed for antibody formation and DTH, and regulatory function of Lyt-1 cells was stressed (21–24). It is conceivable that Lyt-1 preventing cells in our autoimmune system may interact with and activate other T cell subsets in order to suppress the development of self-reactive lymphocytes. The precise sequence of events leading to the suppression of the disease needs to be analyzed.

Several basic concepts have been proposed to explain "self-tolerance" and autoimmunity (for review see ref. 25). One category of such hypotheses assumes that in the normal state lymphocytes capable of recognizing self are deleted or inactivated. Another assumes the existence of self-recognizing lymphocytes that persist in the normal state but are actively suppressed by suppressor T cells. Present analyses of post-thymectomy autoimmune diseases seem to support the second category of ideas, because it is conceivable that diminution of these suppressor cells by NTx may permit the development of self-reactive clones and may allow the emergence of autoimmune disease. Penhale et al. (26) showed that depletion of T cells in rats by ATx followed by a low dose of X-ray irradiations leads to the spontaneous development of autoimmune thyroiditis similar to human Hashimoto's thyroiditis. They also demonstrated that the disease can be prevented by reconstitution with normal lymphoid cells (27). In contrast with Penhale's model and ours, in which autoimmune diseases can be induced without exogenous immunization, the diseases induced by sensitization of experimental animals with autoantigen in adjuvant could be explained as the antigen-triggered expansion of autoreactive cells that overwhelm the regulation by suppressor T cells.

Our post-thymectomy autoimmune model has many features in common with the human organ-specific autoimmune diseases. Thus, it is conceivable that a deficit or dysfunction of the regulatory T cells with similar characteristics may cause human autoimmune disease. Further study of the post-thymectomy autoimmune model may provide the opportunity to elucidate the pathogenesis of these human diseases.

Summary

Autoimmune oophoritis that develops in A/J mice after neonatally thymectomy (NTx) was prevented by a single intraperitoneal injection of spleen cells or thymocytes from normal adult female mice. Prevention of oophoritis was achieved when spleen cells were given within 2 wk after Tx. When spleen cells were obtained from neonatally oophorectomized mice, four times more cells were required for the prevention of oophoritis, but those from the mice oophorectomized on day 7 after birth had equivalent capacity to prevent oophoritis to those from normal female mice.

The spleen cells from normal A/J mice that prevented the development of oophoritis in NTx A/J mice were Thy-1^+, Lyt-1^+,23^-, Ia^-, Qa-1^-, sensitive to in vitro irradiation

with 400 rad, resistant to administration of cyclophosphamide or anti-thymocyte serum, and were not eliminated by adult thymectomy. Thymocytes with oophoritis-preventing capacity were also found to be Lyt-1^+,23^- and TL-1,2,3^-. These results seem to correlate well with the finding that the Lyt-1 subpopulation is substantially decreased in NTx mice.

The results suggest that, in this post-thymectomy autoimmune oophoritis, NTx abrogates the Lyt-1 T cell subpopulation that serves as suppressive or regulatory cells over developing self-reactive cells directed toward ovarian antigens, and eventually may cause autoimmune oophoritis.

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References

1. Nishizuka, Y., and T. Sakakura. 1969. Thymus and reproduction: sex linked dysgenesis of the gonad after neonatal thymectomy in mice. *Science (Wash. D. C.)*. **166:**753.
2. Kojima, A., O. Taguchi, and Y. Nishizuka. 1980. Experimental production of possible autoimmune gastritis followed by macrocytic anemia in athymic nude mice. *Lab. Invest.* **42:**387.
3. Kojima, A., Y. Tanaka-Kojima, T. Sakakura, and Y. Nishizuka. 1976. Spontaneous development of autoimmune thyroiditis in neonatally thymectomized mice. *Lab. Invest.* **34:**550.
4. Taguchi, O., and Y. Nishizuka. 1981. Experimental autoimmune orchitis after neonatal thymectomy in the mouse. *Clin. Exp. Immunol.* **46:**425.
5. Sakakura, Y., and Y. Nishizuka. 1971. Thymic control mechanism in ovarian development: reconstitution of ovarian dysgenesis in thymectomized mice by replacement with thymic and other lymphoid tissues. *Endocrinology.* **90:**431.
6. Kojima, A., Y. Tanaka-Kojima, T. Sakakura, and Y. Nishizuka. 1976. Prevention of postthymectomy autoimmune thyroiditis in mice. *Lab. Invest.* **34:**601.
7. Sakaguchi, S., T. Takahashi, and Y. Nishizuka. 1982. Study on cellular events in postthymectomy autoimmune oophoritis in mice. I. Requirement of Lyt-1 effector cells for oocytes damage following adoptive transfer. *J. Exp. Med.* **156:**1577.
8. Taniguchi, M., T. Saito, and T. Tada. 1979. Antigen-specific suppressive factor produced by a transplantable I-J bearing T cell hybridoma. *Nature (Lond.)*. **278:**555.
9. Boyse, E. A., L. J. Old, and I. Chouroulinkov. 1964. Cytotoxic test for demonstration of mouse antibody. *Methods Med. Res.* **10:**39.
10. Julious, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus derived murine lymphocytes. *Eur. J. Immunol.* **3:**645.
11. Wysocki, L., and V. L. Sato. 1978. Panning for lymphocytes; a method for selection. *Proc. Natl. Acad. Sci. U. S. A.* **75:**2844.
12. Kojima, A., and R. Prehn. 1981. Genetic susceptibility to post-thymectomy autoimmune diseases in mice. *Immunogenetics.* **14:**15.
13. Mathieson, B. J., S. O. Sharrow, P. S. Campbell, and R. Asofsky. 1979. An Lyt differentiated thymocyte subpopulation detected by flow microfluorometry. *Nature (Lond.)*. **277:**478.

14. Potter, T. A., P. M. Hogarth, and I. F. C. McKenzie. 1980. Flow microfluorometric analysis of alloantigen expression during T cell development. *Eur. J. Immunol.* **10:**899.
15. Askenase, P. W., B. J. Hayden, and R. K. Gershon. 1975. Augmentation of delayed type hypersensitivity by doses of cyclophosphamide which do not effect antibody responses. *J. Exp. Med.* **141:**697.
16. Scollay, R., M. Kochen, E. Butcher, and I. Weissman. 1978. Lyt markers on thymus cell migrants. *Nature (Lond.).* **276:**79.
17. Vadas, M. A., J. F. A. P. Miller, I. F. C. McKenzie, S. E. Chism, F.-W. Shen, E. A. Boyse, J. R. Gamble, and A. M. Whitelaw. 1976. Ly and Ia antigen phenotypes of T cells involved in delayed type hypersensitivity and in suppression. *J. Exp. Med.* **144:**10.
18. Liew, F. W., and S. M. Russell. 1980. Delayed type hypersensitivity to influenza virus. Induction of antigen-specific suppressor T cells for delayed type hypersensitivity to hemagglutinin during influenza virus infection in mice. *J. Exp. Med.* **151:**799.
19. Tada, T. 1974. The mode and site of action of suppressor T cells in antigen induced differentiation of B cells. *In* Immunological Tolerance. D. H. Katz and B. Benacerraf, editors. Academic Press, Inc., New York. 471.
20. Herzenberg, L. A., K. Okumura, H. Cantor, V. L. Sato, F.-W. Shen, E. A. Boyse, and L. A. Herzenberg. 1976. T cell regulation of antibody responses: demonstration of allotype-specific helper T cells and their specific removal by suppressor T cells. *J. Exp. Med.* **144:**330.
21. Eardley, D. D., J. Hugenberger, L. McVay-Boudreau, F.-W. Shen, R. K. Gershon, and H. Cantor. 1978. Immunoregulatory circuits among T cell sets to exert feedback inhibition. *J. Exp. Med.* **147:**1106.
22. Sy, M. S., M. H. Diets, R. N. Germain, B. Benacerraf, and M. I. Green. 1980. Antigen and receptor driven regulatory mechanisms. IV. Idiotype-bearing I-J suppressor T cells factors induced second-order suppressor T cells which express anti-idiotypic receptors. *J. Exp. Med.* **151:**1183.
23. Germain, R. N., C. Waltenbaugh, and B. Benacerraf. 1980. Antigen-specific T cell-mediated suppression. V. H-2-linked genetic control of distinct antigen-specific detects in the production and activity of L-glutamic acid^{50}-L-tyrosine^{50} suppressor factor. *J. Exp. Med.* **151:**1243.
24. Cantor, H., L. McVay-Boudreau, J. Hugenberger, K. Naidorf, F.-W. Shen, and R. K. Gershon. 1978. Immunoregulatory circuits among T cells sets. II. Physiologic role of feedback inhibition in vitro: absence in NZB mice. *J. Exp. Med.* **147:**1116.
25. Weigle, W. O. 1980. Analysis of autoimmunity through experimental models of thyroiditis and allergic encephalomyelitis. *Adv. Immunol.* **30:**159.
26. Penhale, W. J., A. Farmer, and W. J. Irvine. 1975. Thyroiditis in T cell-depleted rats: influence of strain, radiation dose, adjuvants and antilymphocyte serum. *Clin. Exp. Immunol.* **21:**362.
27. Penhale, W. J., W. J. Irvine, J. R. Inglis, and A. Farmer. 1976. Thyroiditis in T cell-depleted rats: suppression of the autoallergic response by reconstitution with normal lymphoid cells. *Clin. Exp. Immunol.* **25:**6.