

Estrogen Administration Activates Extrathymic T Cell Differentiation in the Liver

By Ryuhei Okuyama,* Toru Abo, Shuhji Seki,† Toshiaki Ohteki,‡
Keitaro Sugiura,‡ Akinori Kusumi,‡ and Katsuo Kumagai‡

*From the Department of Immunology, Niigata University School of Medicine, Niigata 951; the *Department of Dermatology, Tohoku University School of Medicine; and the †Department of Microbiology, Tohoku University School of Dentistry, Sendai 980, Japan*

Summary

In addition to T cell differentiation in the thymus, we have recently reported that extrathymic T cell differentiation occurs preferentially in the sinusoids of the liver. Although this extrathymic pathway is relatively minor in normal mice, it becomes predominant in mice with autoimmune diseases, athymic mice, and aged mice. In the present study, injection of normal male C3H/He mice, 6–8 wk of age, with 1 mg of estrogen resulted in an increase in mononuclear cells (MNC) yielded from the liver and a drastic decrease in thymocytes approximately 10 d after such injection. This unique modulation was not observed with hydrocortisone injection (5 mg/mouse, i.p.) nor with irradiation (5 Gy/mouse). Rather, these immunosuppressive treatments induced a simultaneous decrease in cell number in both the liver and thymus. A time-kinetics study on the cell number and spontaneous cell proliferation revealed that an increase in spontaneous cell proliferation in the liver preceded the increase in the number of liver MNC, and a decrease in spontaneous cell proliferation in the thymus preceded the decrease in the number of thymocytes. At this time, an enrichment of α/β T cells with intermediate T cell receptors (TCRs), including forbidden T cell oligoclonal and $V\beta 8^+$ cells, which are characterized as extrathymic α/β T cells with unique properties, took place in the liver. On the other hand, the thymic atrophy induced by estrogen resulted in a prominent decrease in immature double-positive ($CD4^+8^+$) α/β T cells with dull TCRs. These results indicate that estrogen administration activates an extrathymic pathway of T cell differentiation in the liver and reciprocally inactivates the intrathymic pathway. As extrathymic T cells have unique characteristics such as autoreactivity, the present findings might be intimately related to a female predominance of autoimmune diseases and suggest a possible role of estrogen in this phenomenon.

Many clinicians and investigators have observed that the female has a greater propensity for the occurrence of various autoimmune diseases than the male. Actual diseases and female-to-male ratios showing a female predominance of autoimmune diseases were reviewed in both humans and animals (1). Although the mechanisms underlying this sex difference are imperfectly understood, it has been suspected that sex hormones might be directly or indirectly associated with this phenomenon. In this respect, estrogen has often been suggested as one of the most probable candidates. For example, abnormal estrogen metabolism resulting in prolonged estrogenic stimulation induced or deteriorated diseased states in SLE patients (2) and administration of oral contraceptives (containing estrogen) to SLE patients exacerbated the disease (3, 4). In experimental animals, estrogen administration was also reported to accelerate autoimmune diseases (5, 6). In parallel with these observations, intensive studies on the immuno-

logical effects of estrogen have been reported (7–10). It was, however, noticed that the effects of estrogen on the immune system so far described did not sufficiently elucidate possible mechanisms of sex hormones involved in the occurrence of autoimmune diseases. It was therein reported that estrogen administration induced profound thymic atrophy and resulted in immunosuppressive effects on the peripheral immune systems. It seems that many difficulties face attempts to clarify the relation between the mechanisms involved in immunosuppression and autoimmune states. Moreover, estrogen administration has been found to sometimes inhibit certain autoimmune diseases, i.e., experimental autoimmune encephalomyelitis (11) and experimental autoimmune thyroiditis (12, 13). It is, therefore, expected that more appropriate or different approaches should be applied to resolve confusion in this area.

It is widely known that T cells differentiate in the thymus

and go through a process of either positive or negative selection to form the repertoires of mature T cells (14–17). In addition to this intrathymic pathway of T cell differentiation, we have recently demonstrated that both α/β and γ/δ T cells may differentiate extrathymically in the liver of humans and mice (18, 19). Although the hepatic pathway of T cell differentiation is relatively minor in normal mice, this pathway becomes predominant in mice subjected to bacterial stimulation (20), in mice with malignancies (21), in autoimmune MRL-*lpr/lpr* mice (18), in congenitally athymic nude mice, and in aged mice with involuted thymus (22). α/β T cells that differentiate in the liver have several unique properties: (a) even forbidden T cell oligoclonal populations could be generated after bacterial stimulation probably due to the lack of the double-positive (DP)¹ CD4⁺8⁺ stage for negative selection (20); (b) V β 8⁺ cells, which are known to be intimately related to various autoimmune disease mice, were preferentially generated (23); and (c) the majority of extrathymic α/β T cells residing in the liver had an intermediate intensity of TCRs (23). It is conceivable that extrathymic α/β T cells may be important in the surveillance of bacterially infected cells and atypical cells generated *in vivo*, and that an overstimulation of such T cells (by undetermined causes) may be responsible for the onset of autoimmune diseases.

The object of the present study was to investigate how estrogen administration affects extrathymic T cell differentiation in the liver. It was demonstrated that estrogen administration might profoundly activate the extrathymic process of T cell differentiation in the liver and, instead, suppress the intrathymic process in the thymus. The possible close relation of the present findings to the mechanisms involved in the female preponderance of autoimmune diseases is discussed.

Materials and Methods

Mice. Male C3H/He mice (H-2^k, Mls-2^a), aged 6–8 wk, were obtained from Charles River Japan Inc. (Tokyo, Japan). They were fed under specific pathogen-free conditions (19, 20).

Estrogen Administration. 1 mg of estrogen (Ovahormone depo; Teikoku Zoki, Inc., Japan)/mouse was subcutaneously injected. As estrogen is dissolved in sesame oil (i.e., due to being water insoluble), only the subcutaneous injection, but not the intravenous or intraperitoneal routes, is available. To investigate the unique effects of estrogen, mice injected intraperitoneally with hydrocortisone (5 mg/mouse; Sigma Chemical Co., St. Louis, MO) and mice irradiated with 5 Gy were also used.

Cell Preparation. Treated and untreated mice were killed, and mononuclear cells (MNC) were prepared from the liver, thymus, and spleen. Liver MNC were obtained as previously described (19, 20). Briefly, to eliminate blood from the liver before its removal, it was perfused with 10 ml PBS (0.01 M, pH 7.2) via the portal vein. To obtain MNC, the liver was pressed through 100-gauge stainless steel mesh and suspended in RPMI 1640 supplemented with 5% FCS. After one washing with the medium, the cells were resuspended in 20 ml of the medium and MNC were isolated from

parenchymal hepatocytes by Ficoll-Isopaque density (1.090) gradient centrifugation. In the MNC preparation method applied here, the proportion of contaminated Kupffer cells was negligible (<4%). The cell yield was ~10–15% (18). MNC of the spleen were also collected by the Ficoll-Isopaque method, whereas thymocytes were obtained by forcing the thymus through 100-gauge mesh (19, 20).

Cell Proliferation Assay. The activity of spontaneous cell proliferation was analyzed by [³H]thymidine uptake into DNA (19). Briefly, freshly isolated MNC (5 × 10⁵ cells/0.2 ml medium) were cultured in a 96-well round-bottomed microplate (Falcon Labware, Oxnard, CA) for 18 h at 37°C in a CO₂ incubator. 0.5 μCi of [³H]thymidine (Amersham International, Amersham, England) was added at the initiation of culture. The medium used here was RPMI 1640 supplemented with 1% fresh mouse serum and 5 × 10⁻⁵ M 2-ME. The data express the mean cpm ± 1 SD of triplicate cultures.

Immunofluorescence Test. Surface phenotypes of cells were identified by using mAbs in conjunction with the single- or two-color immunofluorescence test (18–20). The mAbs used here included biotin-conjugated hamster anti- α/β -TCR (H57-597) (24), FITC-conjugated mouse anti-V β 8 (F23.1) (25), FITC-conjugated hamster anti-CD3 (145-2C11), unconjugated hamster anti-V β 3 (KJ25-606-4) (26), and biotin-conjugated IL-2R β chain (TM- β 1) (kindly provided from Drs. T. Tanaka and M. Miyasaka, The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan) mAbs (27). Biotin-conjugated reagent was developed with PE-conjugated avidin, and unconjugated reagent was developed with PE-conjugated goat anti-hamster Ig (Caltag Laboratories, San Francisco, CA). FITC-conjugated rat anti-CD4 (L3T4) and PE-conjugated anti-CD8 (Lyt2) mAbs were also used (Becton Dickinson & Co., Mountain View, CA). The fluorescence-positive cells were analyzed by a FACScan[®] (Becton Dickinson & Co.).

Results

Unique Effects of Estrogen on the Number of MNC in the Liver and Thymus. When C3H/He mice were subcutaneously injected with estrogen (1 mg/mouse), a prominent increase of the number of MNC obtained from the liver was demonstrated 10 d after the injection (Fig. 1). In contrast, severe thymic atrophy and resulting decrease in the number of thymocytes occurred at the same time. To emphasize this unique effect of estrogen, we also examined the number of MNC in the liver and thymus of mice injected with hydrocortisone (5 mg/mouse) or of mice irradiated with 5 Gy, which are known to be representative immunosuppressive treatments. After 5 d of these treatments, simultaneous decreases in the number of MNC in the liver and thymus occurred. The suppressive effect of hydrocortisone (5 mg/ml) was induced by both intraperitoneal and subcutaneous routes of injection (our unpublished observation). The maximum suppression was also achieved by both routes 5 d after the injection.

Time-Kinetics of Variations in the Number of MNC in the Liver, Thymus, and Spleen after Estrogen Administration. Precise time-kinetics of variations in the number of MNC was then analyzed (Fig. 2). After 5 d of estrogen administration, the number of MNC in the liver began to increase, reached a peak on day 13, and then decreased gradually. The variation in the number of thymocytes appeared to be completely reversed, since the number of thymocytes began to decrease 3 d after

¹ Abbreviations used in this paper: DN, double negative; DP, double positive; MNC, mononuclear cells.

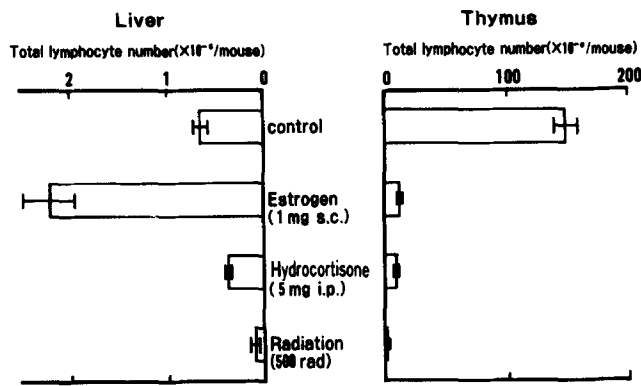


Figure 1. Variation of the number of MNC in the liver and thymus of mice treated with estrogen, hydrocortisone, and irradiation. Four mice were subcutaneously injected with estrogen (1 mg/mouse) and killed 10 d after the injection, whereas four mice were intraperitoneally injected with hydrocortisone (5 mg/mouse) and killed 5 d after the injection. Four irradiated mice (5 Gy/mouse) were also used 5 d after the treatment. Data express mean \pm 1 SD.

the injection, reached a base line on day 7, and then showed a gradual restoration 15 d after the injection. A pattern of variation similar to that seen in the thymus was also observed in the spleen, although the variation was not so drastic as that in the thymus.

Reciprocal Change between the Liver and Thymus Was also Observed in Spontaneous Cell Proliferation. In contrast to lymphocytes obtained from the peripheral immune organs, spleen, and lymph nodes, thymocytes and freshly isolated hepatic MNC often showed increased spontaneous cell proliferation in *in vitro* culture (18, 21). This spontaneous cell proliferation was serially analyzed in the liver and thymus after estrogen administration (Fig. 3). Interestingly, an increase in spontaneous proliferation of the hepatic MNC was observed to coincide with the period when the number of MNC increased. On the other hand, the spontaneous proliferation of thymocytes disappeared coincident with the time when

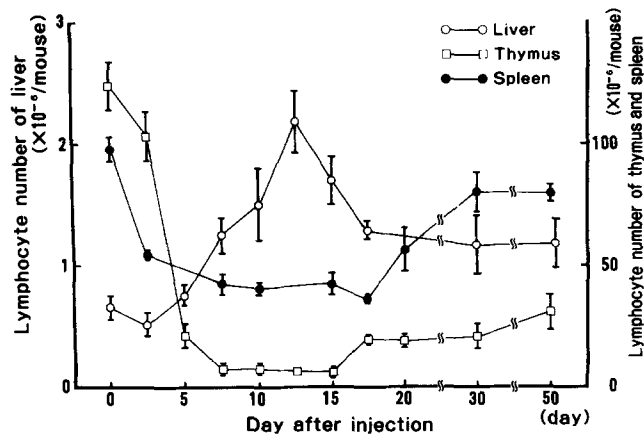


Figure 2. Time-kinetics of variations in MNC number of the liver, thymus, and spleen after estrogen administration. Each plot was based on the data of four individual mice tested.

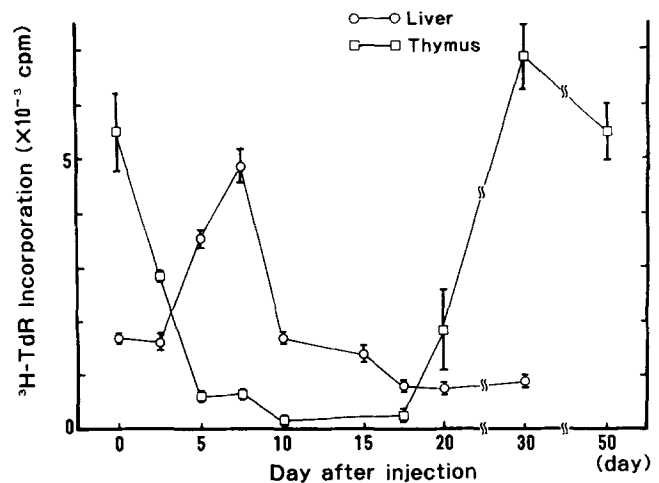


Figure 3. Time-kinetics of variation in the spontaneous proliferation of liver MNC and thymocytes after estrogen administration. Each plot was based on the data of four individual mice treated.

the number of thymocytes was at the base line. Strictly speaking, an increase in the spontaneous cell proliferation in the liver seemed to slightly precede the increase in the number of MNC, whereas a decrease in the spontaneous cell proliferation in the thymus seemed to precede the decrease in the number of thymocytes.

Specific Activation of α/β T Cells with Intermediate TCR in the Liver. It was previously reported that MNC in the liver contained a large population of α/β T cells with either intermediate TCR or bright TCR (20, 23). The staining pattern of TCR, V β 8, CD3, and V β 3 antigens was analyzed in the liver MNC before and after the injection (Fig. 4). It was confirmed that α/β T cells in the liver consisted of both intermediate TCR (or CD3) and bright TCR (or CD3). The former cells were a relatively minor population in the liver as compared with the latter cells in untreated control mice. 10 d after injection, almost all T cells were composed of intermediate TCR. This pattern was quite similar to that seen in the liver of autoimmune MRL-*lpr/lpr* mice and thymectomized mice as we previously reported (23).

In a recent study, we developed the most accurate method to identify intermediate TCR cells by a two-color immunofluorescence test using anti-CD3 and anti-IL-2R β mAbs (H. Watanabe, manuscript in preparation). By applying this method, we investigated the kinetics of estrogen dose and the time course in hepatic MNC (Fig. 5). The results on days 5 and 10 are represented and the doses used here were 0.125, 0.25, 0.5, and 1 mg/mouse of estrogen. We noticed that the time course after estrogen administration showed the delayed onset (approximately several days after the injection) and long-lasting effect, especially at the high doses, were probably due to the effect of the oil solvent or the intrinsic property of estrogen. As intermediate TCR cells (encompassed by squares in Fig. 5), as well as CD3⁻ NK cells, expressed primarily the high level of IL-2R β , the peak of intermediate TCR cells (18.2%) was clearly demonstrated even in untreated mice.

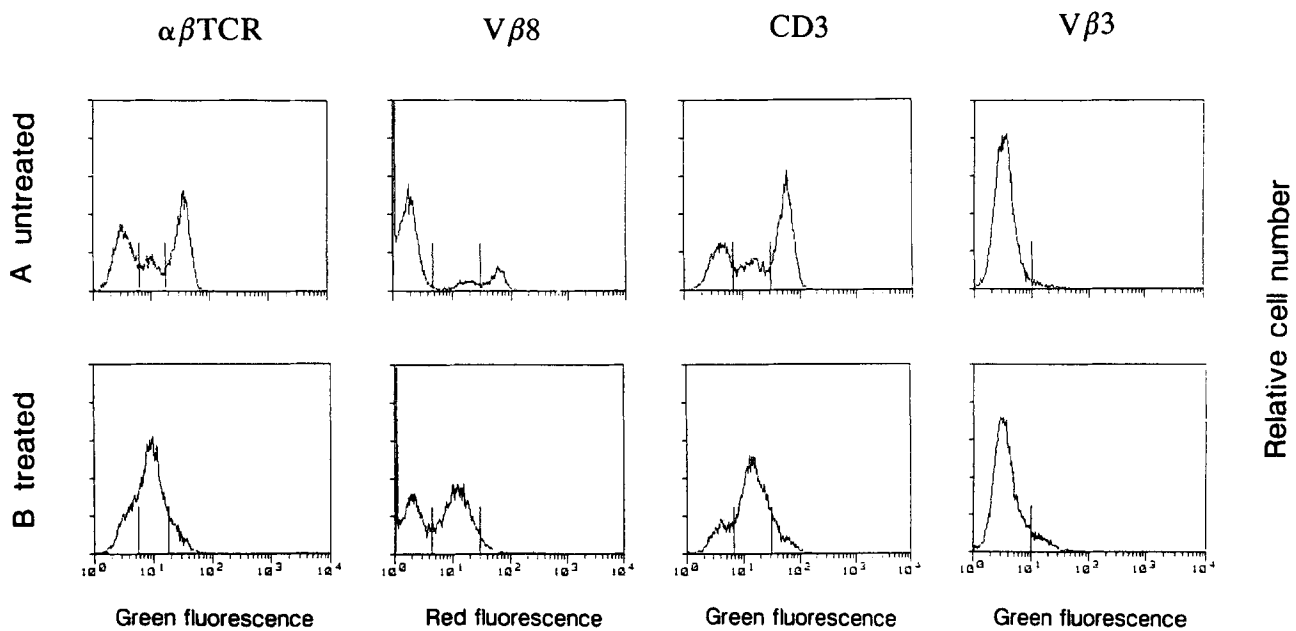


Figure 4. Specific activation of α/β T cells with intermediate TCRs in the liver. A representative result of four isolated experiments is shown.

This proportion was increased as a function of estrogen dose up to >50%. The low doses of estrogen (0.125 and 0.25 mg/mouse) showed a relatively small effect on day 10. After an application of this accurate method, we know that the actual proportion of intermediate α/β -TCR cells in the liver is lower than those identified by the single-color method.

An Increase of $V\beta 8^+/\alpha/\beta$ -TCR⁺ Ratios and Generation of Forbidden T Cell Oligoclones after Estrogen Administration. We then investigated whether the increase of α/β T cells with intermediate TCR in the liver after estrogen administration was accompanied by the generation of $V\beta 8^+$ cells and forbidden T cell clones (Table 1). It was demonstrated that α/β

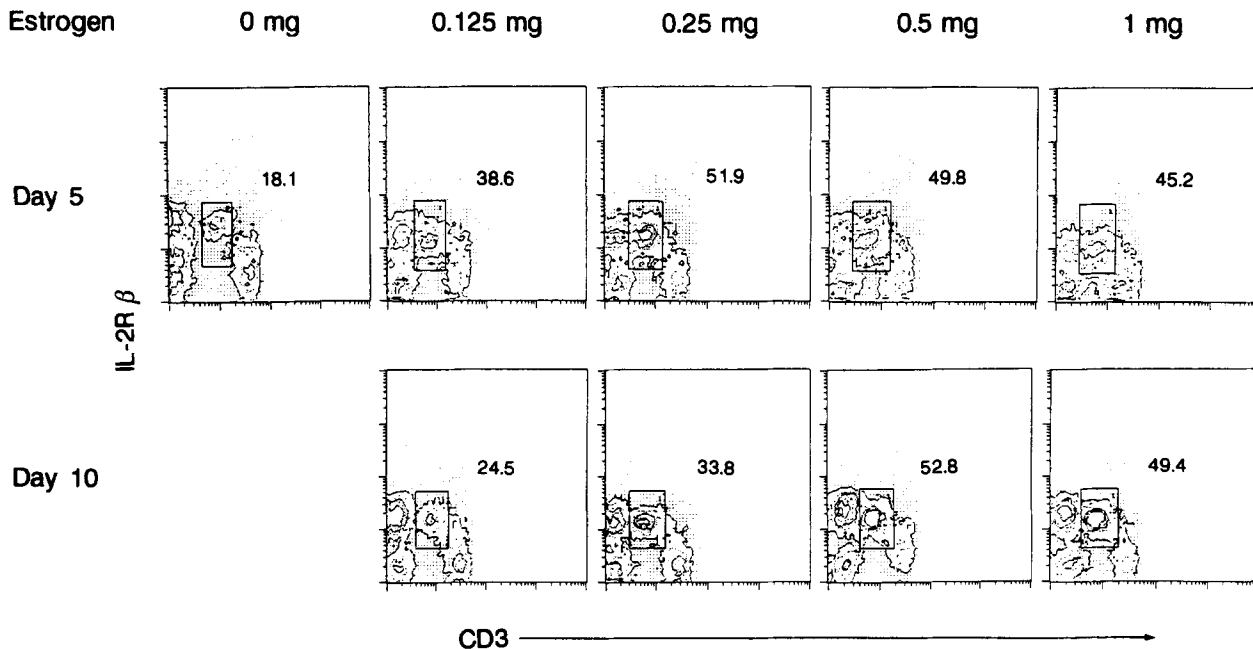


Figure 5. FACS profiles of a two-color immunofluorescence test: the identification of intermediate TCR cells and the proportional increase by estrogen administration as a function of dose. Here, intermediate TCR cells (encompassed by squares) were identified by the two-color immunofluorescence test using anti-CD3 and anti-IL-2R β mAbs.

Table 1. Phenotypic Analysis of Liver MNC before and after Estrogen Administration

Cell marker	Intensity of TCR	Percent fluorescence-positive cells	
		-	Estrogen
α/β -TCR ⁺	Intermediate	15.3 ± 2.3	63.1 ± 5.2
	Bright	46.4 ± 3.4	15.5 ± 0.3
V β 8 ⁺	Intermediate	8.1 ± 0.3	50.3 ± 4.7
	Bright	12.8 ± 0.4	5.0 ± 0.1
CD3 ⁺	Intermediate	18.8 ± 0.4	79.8 ± 5.8
	Bright	52.8 ± 4.8	6.3 ± 0.1
V β 8 ⁺ / α/β -TCR ⁺	Intermediate	52.9 ± 3.4	79.7 ± 4.6
	Bright	27.6 ± 2.8	32.3 ± 2.9

Five mice were individually examined, and the mean and 1 SD are represented.

T cells with intermediate TCR (or CD3) became predominant after estrogen administration and that such intermediate TCR cells contained abundant V β 8⁺ cells (79.8 ± 5.8%; *n* = 5). Even before estrogen administration, intermediate TCR cells in the liver contained a considerably high proportion of V β 8⁺ cells (52.9 ± 3.4%), whereas bright TCR cells showed a constantly low level (~30%) of V β 8⁺ cells irrespective of estrogen administration.

The proportion of V β 3⁺ cells, which are reactive to Mls-2^a and are therefore one of the forbidden T cell oligo clones for C3H/He (Mls-2^a) mice, was also analyzed in the liver before and after estrogen administration (see Fig. 4, right, and Table 2). As expected, V β 3⁺ cells were a negligible proportion (<1%) in all immune organs tested before estrogen administration. On the other hand, a significant proportion of V β 3⁺ cells (8.9 ± 1.2%; *n* = 4) appeared only in the liver after injection. α/β T cells with intermediate

Table 2. Generation of V β 3⁺ Forbidden T Cell Clone in the Liver after Estrogen Injection

Organ	Percent V β 3 ⁺ forbidden T cell clone	
	-	Estrogen
Liver	0.5 ± 0.1	8.9 ± 1.2
Thymus	0.1 ± 0	0.9 ± 0.2
Spleen	0.9 ± 0.2	2.2 ± 0.4
Lymph nodes	0.4 ± 0.1	0 ± 0

Four mice were individually examined, and the mean and 1 SD are represented.

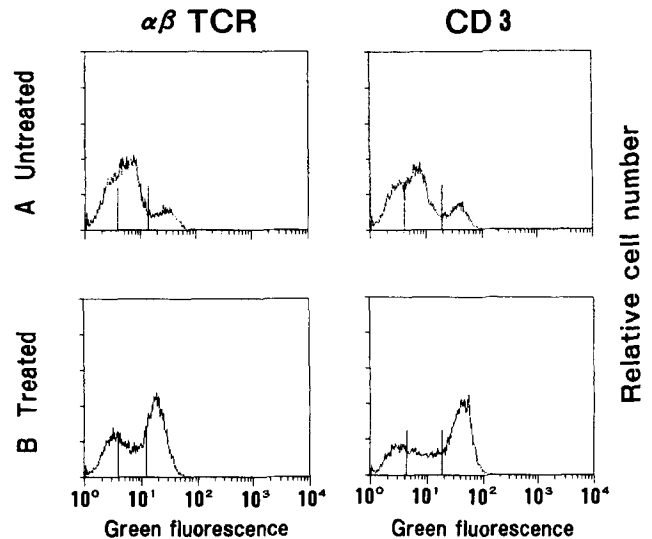


Figure 6. Decrease of dull TCR cells in the thymus after estrogen administration. A representative result of four isolated experiments is shown.

TCR also appeared only in the liver, and V β 3⁺ cells were shown to reside in this fraction.

Disappearance of DP α/β T Cells with Dull TCR in the Thymus after Estrogen Administration. It is well established that α/β T cells in the thymus consist of both dull TCR cells and bright TCR cells (14–17). The former cells are mainly immature DP CD4⁺8⁺ cells before a negative or positive selection process, and the latter cells are mature single-positive CD4⁺ or CD8⁺ cells after the selection process. As thymic atrophy and the resulting thymocytopenia were the most prominent findings in mice injected with estrogen, we finally investigated what type of cells specifically decreased at this time (Fig. 6). A drastic decrease in the proportion of dull TCR cells was demonstrated by the staining of both α/β -TCR and CD3. Although the relative proportion of bright TCR cells in the thymus increased after estrogen treatment, calculation showed the absolute number of such bright TCR cells was also shown to decrease (up to 1:5).

A two-color immunofluorescence test of CD4 and CD8 staining in the thymus was then analyzed (Fig. 7). As anticipated, DP CD4⁺8⁺ cells selectively decreased (80.5 to 35.0%) after a single injection of estrogen. This fraction corresponded to dull TCR cells in the former experiment. If estrogen administration was repeated more than twice every other day, the proportion of DP CD4⁺8⁺ cells decreased to the basal level (<1%) (data not shown).

Discussion

In the present study, we demonstrated that estrogen administration stimulated extrathymic T cell differentiation in the liver of mice in terms of the increased MNC number in the liver and an induced predominance of α/β T cells with intermediate TCRs. These α/β T cells contained V β 3⁺ (reactive to Mls-2^a) forbidden T cell oligoclonal for these

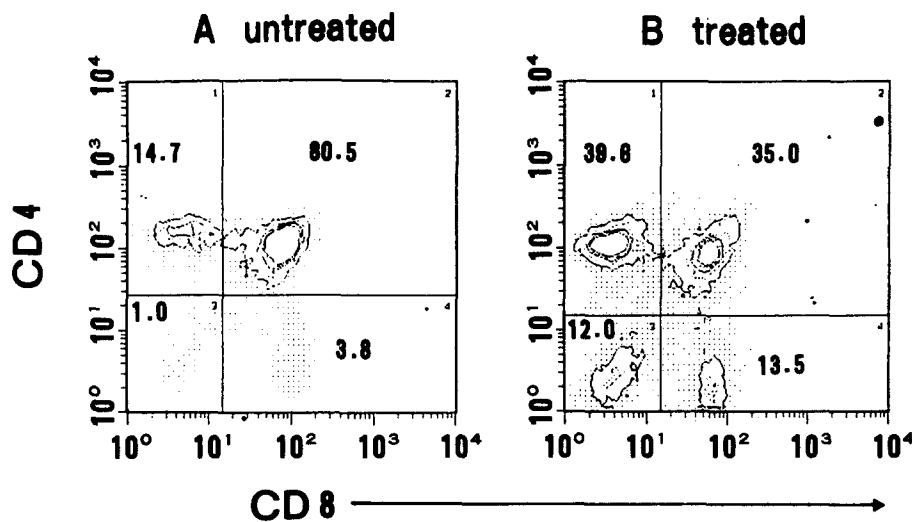


Figure 7. Two-color immunofluorescence test of CD4 and CD8 staining in the thymus. A representative result of four isolated experiments is shown. Numbers in the figure express the percentages of fluorescence-positive cells in the squares.

mice, and $V\beta 8^+$ T cells, both of which were, although not entirely, previously characterized as extrathymic T cells with unique properties seen in the liver of mice under autoimmune conditions (18, 23) and malignancies (19, 21). On the other hand, estrogen administration appeared to suppress intrathymic T cell differentiation in terms of the decreased thymocyte number and the disappearance of immature DP $CD4^+ 8^+$ α/β T cells with dull TCRs. As an overactivation of the extrathymic pathway of T cell differentiation might be responsible for a possible induction or acceleration of autoimmune diseases, the present findings might elucidate the immunological basis of the mechanisms involved in the female predominance of autoimmune diseases.

Several investigators have proposed the existence of an extrathymic pathway for T cell differentiation in experiments utilizing congenitally athymic nude mice (28–30) and in vitro culture systems (31, 32). However, a consensus has not yet been reached, since there is no definite information as to where such T cells differentiate outside the thymus. We have recently reported that such α/β (and γ/δ) T cells differentiate preferentially in the sinusoids of the liver, especially under conditions of autoimmune diseases (18, 23), malignancies (19, 21), bacterial stimulations (20), and aging (22). The extrathymic α/β T cells generated in the liver have several unique properties not seen in those differentiated in the thymus. In the liver as well as in the thymus, α/β T cells show a two-peak pattern of dull and bright TCR expression (including CD3 antigens) in terms of immunofluorescence staining intensity (20). A single-peak pattern of only bright α/β -TCR and CD3 is seen in other peripheral immune organs, including the spleen, lymph nodes, peripheral blood, intestine, and skin. However, the peak of hepatic dull TCR should be estimated as “intermediate” because it is somewhat brighter than thymic dull TCR (23). As thymectomized mice (after 2 mo of thymectomy) had only α/β T cells with intermediate TCR in the liver, we have postulated that such α/β T cells are a major population of extrathymic α/β T cells. Indeed, congenitally athymic mice have α/β T cells with intermediate TCR in

not only the liver but also in the peripheral immune organs, spleen, and lymph nodes (33).

α/β T cells with intermediate TCR seen in the liver are comprised of either double-negative (DN) $CD4^- 8^-$ cells or single-positive ($CD4^+$ or $CD8^+$) cells, and absolutely lack DP $CD4^+ 8^+$ cells (18, 20, 23). It is therefore, conceivable that extrathymic α/β T cells lack a process of negative selection and that forbidden T cell clones are therefore generated in the liver. In addition, only hepatic α/β T cells with intermediate TCRs comprise a high proportion (>60%) of $V\beta 8^+$ cell oligoclonal (normal values are $\sim 30\%$) (23). $V\beta 8^+$ cells have recently been well characterized as the cells responsible for the onset of autoimmune disease due to their autoreactivity (34). Their roles in the onset of diseases in autoimmune mice, e.g., MRL-*lpr/lpr* mice (23), mice with experimental autoimmune encephalomyelitis (35, 36), and diabetic NOD mice (37), have been reported.

Concerning the above evidence from our recent studies, we prefer to consider that estrogen administration activates an extrathymic pathway of T cell differentiation in the liver. Our subsequent study further demonstrated that repeated injections of lower doses of estrogen could induce continuous activation of the hepatic pathway of T cell differentiation (unpublished observation). It is conceivable that such activated, extrathymic α/β T cells, which contain a large population of $V\beta 8^+$ cells and considerable proportions of forbidden T cell oligoclonal, may be responsible for the induction or acceleration of certain autoimmune diseases.

We recently examined the estrogen effects on the extrathymic T cell differentiation in the liver of athymic nude BALB/c-*nu/nu* mice aged 8 and 20 wk (data not shown). In contrast to our expectation, we could not produce a significant augmentation of extrathymic T cell differentiation by estrogen (1 mg/mouse, s.c.) in terms of changes in the number of hepatic MHC and the proportion of intermediate TCR cells. Although we will report that athymic nude mice have only intermediate TCR cells in the liver and, to some extent, in the periphery, the absolute number of such

cells is not high (33). It is probably that nude mice may not have the best microenvironments even for the extrathymic T cell differentiation in the liver.

We also confirmed a drastic decrease of thymocyte number after estrogen administration. Many earlier studies on the immunological effects of estrogen demonstrated a rapid induction of thymic atrophy and the subsequent decrease of T lymphocytes in the peripheral immune organs (8, 38–40). In the present study, we clearly showed that such a rapid decrease in the number of thymocytes was mainly caused by the selective disappearance of immature DP CD4⁺8⁺ α/β T cells with dull TCR. We have further demonstrated that the disappearance of each DP α/β T cell was due to an acceleration of the apoptosis normally seen in the thymus (our unpublished observation). It is noteworthy that activation of extrathymic T cell differentiation in the liver is always accompanied by an inactivation of intrathymic T cell differentiation, i.e., aging (22), malignancies (21), bacterial stimulations (20), and estrogen administration. It is conceivable that the extrathymic and intrathymic pathways for T cell differentiation may be reciprocally regulated by undetermined factors. The mechanisms underlying this phenomenon are under investigation. We have recently noticed that estrogen administration may activate stromal cells in the thymus (i.e., thymic epithelial cells) and possibly stromal cells in the liver (i.e., sinusoidal endothelial cells and Kupffer cells), probably via their estrogen receptors on the cell surface (41, 42).

There were reports that estrogen administration inhibited the onset of certain autoimmune diseases (11–13), i.e., experimental autoimmune encephalomyelitis and thyroiditis. In contrast to the spontaneous onset of many autoimmune diseases, those experimental diseases were caused by the injection of foreign antigens (even if they mimic autoantigens).

It is conceivable that at least during the period of onset, such experimental diseases might be important for α/β T cells generated intrathymically. If this is the case, estrogen administration is expected to act as an inhibitory factor due to its suppressive effect on the intrathymic pathway of T cell differentiation. Our present findings may explain some of the conflicting effects of estrogen administration on autoimmune diseases.

A question is raised as to how estrogen administration is related to autoantibody production seen in autoimmune diseases. One possibility is that extrathymic DN CD4⁻8⁻ (or single-positive) α/β T cells with intermediate TCRs have a helper function of certain B cells (e.g., CD5⁺ B cells). Certain DN α/β T cells have already been reported to be associated with the enhancement of autoantibody production by B cells (43, 44). In the autoimmune mice used in such studies, extrathymic α/β T cells with intermediate TCRs may be in activated states in the liver. The interaction of such extrathymic α/β T cells with certain B cells remains to be further investigated. At least, estrogen administration is known to augment antibody production of thymus-independent antigens (1, 45). Extrathymic T cells may be a candidate in this case. In any case, the present results have led us to consider the possibility that physiological and pharmacological doses of estrogen might activate an extrathymic pathway for T cell differentiation. Such extrathymic α/β T cells might be beneficial for the surveillance of atypical cells generated in vivo due to their autoreactivity. However, if an overstimulation of this pathway occurs in individuals with certain genetic or other traits, they may fall victim to autoimmune diseases. In such cases, it is probable that estrogen may act as one of the factors in the female preponderance of autoimmune diseases.

We thank Mrs. Masako Watanabe for manuscript preparation.

This work was supported by a Grant-in-Aid for Scientific Research and Cancer Research from the Ministry of Education, Science and Culture, Japan, and by a Grant-in-Aid from the Mochida Memorial Foundation for Medical and Pharmaceutical Research, Japan.

Address correspondence to Toru Abo, Department of Immunology, Niigata University School of Medicine, Asahimachidori 1-757, Niigata 951, Japan.

Received for publication 28 May 1991 and in revised form 21 November 1991.

References

1. Ahmed, S.A., W.J. Penhale, and N. Tälal. 1985. Sex hormones, immune responses, and autoimmune diseases. Mechanisms of sex hormone action. *Am. J. Pathol.* 121:531.
2. Lahita, R.G., H.L. Bradlow, H.G. Kunkel, and J. Fishman. 1979. Alteration of estrogen metabolism in systemic lupus erythematosus. *Arthritis Rheum.* 22:1195.
3. Chapel, T.A., and R.E. Burns. 1971. Oral contraceptives and exacerbations of lupus erythematosus. *Am. J. Obstet. Gynecol.* 110:366.
4. Jungers, P., M. Dougados, C. Pelissier, K. Nahoul, F. Tron, and J.-F. Bach. 1982. Influence of oral contraceptive therapy on the activity of systemic lupus erythematosus. *Arthritis Rheum.* 25:618.
5. Roubinian, J.R., N. Tälal, J.S. Greenspan, J.R. Goodnan, and K. Siiteri. 1978. Effect of castration and sex hormone treatment survival, anti-nucleic acid antibodies, and glomerulone-

- phritis in NZB × NZW F1 mice. *J. Exp. Med.* 147:1568.
6. Roubinian, J., N. Talal, P. Siiteri, and J.A. Sadakian. 1979. Sex hormone modulation of autoimmunity in NZB/NZW mice. *Arthritis Rheum.* 22:1162.
 7. Stern, K., and I. Davidsohn. 1955. Effect of estrogen and cortisone on immune hemoantibodies in mice of inbred strains. *J. Immunol.* 74:479.
 8. Thompson, J.S., M.K. Crawford, R.W. Reilly, and C.D. Severson. 1967. The effect of estrogenic hormones on immune responses in normal and irradiated mice. *J. Immunol.* 108:331.
 9. Ablin, R.J. 1981. Modulatory effects of estrogen on immunologic responsiveness. *Am. J. Reprod. Immunol.* 1:206.
 10. Ablin, R.J., and T. Kallard. 1984. Immunomodulatory effects of estrogen. *J. Immunol.* 132:3229.
 11. Arnason, B.G., and D.P. Richman. 1969. Effect of oral contraceptives on experimental demyelinating disease. *Arch. Neurol.* 21:103.
 12. Kappas, A., H.E.H. Jones, and I.M. Roitt. 1963. Effects of steroid sex hormones on immunological phenomenon. *Nature (Lond.)* 198:902.
 13. Okayasu, I., Y.M. Kong, and N.R. Rose. 1981. Effect of castration and sex hormones on experimental autoimmune thyroiditis. *Clin. Immunol. Immunopathol.* 20:240.
 14. Kisielow, P., H. Blüthmann, U.D. Staerz, M. Steinmetz, and H. von Boehmer. 1988. Tolerance in T-cell-receptor transgenic mice involves deletion of nonmature CD4⁺8⁺ thymocytes. *Nature (Lond.)* 333:742.
 15. Teh, H.S., P. Kisielow, B. Scott, H. Kishi, Y. Uematsu, H. Blüthmann, and H. von Boehmer. 1988. Thymic histocompatibility complex antigens and the α/β T-cell receptor determine the CD4/CD8 phenotype of T cells. *Nature (Lond.)* 335:229.
 16. Smith, C.A., G.T. William, R. Kingston, E.J. Jenkinson, and J.J.T. Owen. 1989. Antibodies to CD3/T-cell receptor complex induce death by apoptosis in immature T cells in thymic cultures. *Nature (Lond.)* 337:181.
 17. Finkel, T.H., J.C. Cambler, R.T. Kubo, W.K. Born, P. Marrack, and J.W. Kappler. 1989. The thymus has two functionally distinct populations of immature $\alpha\beta$ T cells: one population is deleted by ligation of $\alpha\beta$ TCR. *Cell.* 58:1047.
 18. Ohteki, T., S. Seki, T. Abo, and K. Kumagai. 1990. Liver is a possible site for the proliferation of abnormal CD3⁺4⁻8⁻ double-negative lymphocytes in autoimmune MRL-*lpr/lpr* mice. *J. Exp. Med.* 172:7.
 19. Seki, S., T. Abo, T. Masuda, T. Ohteki, A. Kanno, K. Takeda, H. Rikiishi, H. Nagura, and K. Kumagai. 1990. Identification of activated T cell receptor $\gamma\delta$ lymphocytes in the liver of tumor-bearing hosts. *J. Clin. Invest.* 86:409.
 20. Abo, T., T. Ohteki, S. Seki, N. Koyamada, Y. Yoshikai, T. Masuda, H. Rikiishi, and K. Kumagai. 1991. The appearance of T cells bearing self-reactive T cell receptor in the livers of mice injected with bacteria. *J. Exp. Med.* 174:417.
 21. Seki, S., T. Abo, K. Sugiura, T. Ohteki, T. Kobata, H. Yagita, K. Okumura, H. Rikiishi, T. Masuda, and K. Kumagai. 1991. Reciprocal T cell responses in the liver and thymus of mice injected with syngeneic tumor cells. *Cell. Immunol.* 137:46.
 22. Ohteki, T., T. Abo, S. Seki, T. Kobata, H. Yagita, K. Okumura, and K. Kumagai. 1991. Predominant appearances of $\gamma\delta$ T lymphocytes in the liver of mice after birth. *Eur. J. Immunol.* 21:1733.
 23. Seki, S., T. Abo, T. Ohteki, K. Sugiura, and K. Kumagai. Unusual $\alpha\beta$ T cells expanded in autoimmune *lpr* mice are probably a counterpart of normal T cells in the liver. *J. Immunol.* 147:1214.
 24. Kubo, T.R., W. Born, J.W. Kappler, P. Marrack, and M. Pigeon. 1989. Characterization of a monoclonal antibody which detects all murine T cell receptors. *J. Immunol.* 142:2736.
 25. Staerz, U.D., H.-G. Rammensee, J.D. Benedetto, and M.J. Bevan. 1985. Characterization of a murine monoclonal antibody specific for an allotypic determinant on T cell antigen receptor. *J. Immunol.* 134:3994.
 26. Abe, R., M.S. Vacchio, B. Fox, and R.J. Hodes. 1988. Preferential expression of the T-cell receptor V β 3 gene Mls^c reactive T cells. *Nature (Lond.)* 335:827.
 27. Tanaka, T., M. Tsudo, H. Karasuyama, F. Kitamura, T. Kono, M. Hatakeyama, T. Taniguchi, and M. Miyasaka. 1991. A novel monoclonal antibody against murine IL-2 receptor β chain: characterization of receptor expression in normal lymphoid cells and EL4 cells. *J. Immunol.* 147:2222.
 28. Yoshikai, Y., M.D. Reis, and T.W. Mak. 1986. Athymic mice express a high level of functional gamma-chain but greatly reduced levels of alpha- and beta-chain T-cell receptor messages. *Nature (Lond.)* 324:482.
 29. Kishihara, K., Y. Yoshikai, G. Matsuzaki, T.W. Mak, and K. Nomoto. 1987. Functional alpha and beta T cell chain receptor messages can be detected in old but not young athymic mice. *Eur. J. Immunol.* 17:477.
 30. Pardoll, D.M., B.J. Fowlkes, A.M. Lew, W.L. Maloy, M.A. Weston, J.A. Bluestone, and R.H. Schwartz. 1988. Thymus-dependent and thymus-independent development pathways for peripheral T cell receptor-gamma delta-bearing lymphocytes. *J. Immunol.* 140:4091.
 31. Preffer, F.I., C.W. Kim, K.H. Fisher, E.M. Sabga, R.L. Kradin, and R.B. Colvin. 1989. Identification of pre-T cells in human peripheral blood. Extrathymic differentiation of CD7⁺3⁻ cells into CD3⁺ γ/δ ⁺ or α/β ⁺ T cells. *J. Exp. Med.* 170:177.
 32. Abo, T., S. Sugawara, S. Seki, M. Fujii, H. Rikiishi, K. Takeda, and K. Kumagai. 1990. Induction of human TCR $\gamma\delta$ ⁺ and TCR $\gamma\delta$ ⁻ CD2⁺CD3⁻ double negative lymphocytes by bacterial stimulation. *Int. Immunol.* 2:775.
 33. Watanabe, H., K. Ohtsuka, M. Kimura, Y. Ikarashi, T. Ohmori, A. Kusumi, T. Ohteki, S. Seki, and T. Abo. 1991. Details of an isolation method for hepatic lymphocytes in mice. *J. Immunol. Methods.* In press.
 34. Seman, M., S. Boudaly, T. Roger, J. Morisset, and G. Pham. 1990. Autoreactive T cells in normal mice: unrestricted recognition of self peptides on dendritic cell I-A molecules by CD4⁻8⁻ T cell receptor α/β ⁺ T cell clones expressing V β 8.1 gene segments. *Eur. J. Immunol.* 20:1265.
 35. Zamvil, S.S., D.J. Mitchell, N.E. Lee, A.C. Moore, M.K. Waldor, K. Sakai, J.B. Rothbard, H.O. McDevitt, L. Steinman, and H.A. Orbea. 1988. Predominant expression of a T cell receptor V β gene subfamily in autoimmune encephalomyelitis. *J. Exp. Med.* 167:1586.
 36. Vandenbark, A.A., G. Hashim, and H. Offner. 1989. Immunization with a synthetic T-cell receptor V-region peptide protects against experimental autoimmune encephalomyelitis. *Nature (Lond.)* 341:541.
 37. Bacej, A., B. Charlton, and T.E. Mandel. 1989. Prevention of cyclophosphamide-induced diabetes by anti-V β 8 T-lymphocyte-receptor monoclonal antibody therapy in NOD/Whei mice. *Diabetes.* 38:1492.
 38. Chiodi, H. 1940. The relationship between the thymus and the sexual organs. *Endocrinology.* 26:107.
 39. Sobhon, P., and C. Jirasatham. 1974. Effect of sex hormones on the thymus and lymphoid tissue of ovariectomized rats. *Acta Anat.* 89:211.

40. Ahmed, S.A., M.J. Dauphinee, and N. Talal. 1985. Effects of short term administration of sex hormones on normal and autoimmune mice. *J. Immunol.* 134:204.
41. Grossman, C.J., L.J. Sholiton, and J.A. Helmsworth. 1983. Characteristics of the cytoplasmic and nuclear dihydrotestosterone receptors of human thymic tissue. *Steroids.* 42:11.
42. Dulk, M.M.C.D., R.W. Crofton, and R. VanFurth. 1979. Origin and kinetics of Kupffer cells during an acute inflammatory response. *Immunology.* 37:7.
43. Sainis, K., and S.K. Datta. 1988. CD4⁺ T cell lines with selective patterns of autoreactivity as well as CD4⁻CD8⁻ T helper cell lines augment the production of idiotypes shared by pathogenic anti-DNA autoantibodies in the NZB × SWR model of lupus nephritis. *J. Immunol.* 140:2215.
44. Datta, S.K., H. Patel, and D. Berry. 1987. Induction of a cationic shift in IgG anti-DNA autoantibodies. Role of T helper cells with classical and novel phenotypes in three murine models of lupus nephritis. *J. Exp. Med.* 165:1252.
45. Brick, J.E., D.A. Wilson, and S.E. Walker. 1985. Hormonal modulation of response to thymus-independent and thymus-dependent antigens in autoimmune NZB/W mice. *J. Immunol.* 134:3693.