Demonstration of Large-Scale Migration of Cortical Thymocytes to Peripheral Lymphoid Tissues in Cyclosporin A–treated Rats

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Summary

Young adult Lewis rats were maintained on diets containing 0.015 or 0.027% cyclosporin A (CSA) for periods of up to 6 wk. All animals showed complete depletion of medullary thymocytes ($CD4^+8^-$ and $CD4^-8^+$, T cell receptor [TCR] α/β^{hi}, Thy-$1^{med/low}$, terminal deoxynucleotidyl transferase negative [TdT^-]) and a 50% reduction in the number of TdT^- cortical thymocytes ($CD4^+8^+$, TCR α/β^{low}, Thy-1^{med}) within 1 wk of CSA treatment. In addition, about half of the animals displayed a 50% reduction in the number of TdT^+ cortical thymocytes ($CD4^+8^+$, TCR α/β^{low}, Thy-1^{hi}). These intrathymic changes were accompanied by a reciprocal increase in the number of double-positive (DP; $CD4^+8^+$) T cells in lymph nodes (LN) and spleens. To confirm that the latter T cells were recent thymic emigrants (RTE), CSA-treated rats were injected intrathymically with fluorescein isothiocyanate, and the phenotype of the labeled T cells appearing in LN was determined 16 h later. The results demonstrated that, in addition to those RTE exported in normal animals (>90% medullary origin), the emigration of DP thymocytes, including large numbers of TdT^+ thymocytes, was markedly increased. The presence of TdT^+ cells, which normally do not leave the thymus, clearly identifies the DP RTE as originating from the thymus cortex. Intrathymic labeling studies also directly demonstrated that export of all thymocyte subsets ceases within 9 d of CSA treatment; and thymectomy experiments confirmed that the CSA-induced increase in phenotypically immature T cells resulted primarily from the disturbance of thymocyte maturation and emigration, rather than from a direct effect on preexisting T cells. These results suggest that a wave of cortical thymocytes, many of which presumably have not yet undergone negative selection, is released from the thymus during the first week of CSA treatment. The presence of these potentially unselected cells in peripheral lymphoid tissues may help to explain the increased frequency of autoreactive T cells observed in CSA-treated animals.

Cyclosporin A (CSA)[1] is a product of fungal metabolism that has very useful immunosuppressive properties clinically in the prevention of organ graft rejection and GVHD, and in the treatment of autoimmune disorders (reviewed in references 1 and 2). However, under certain circumstances CSA has the paradoxical effect of promoting syngeneic GVHD and organ-specific autoimmunity. Thus, CSA treatment after irradiation and syngeneic bone marrow transplantation produces severe GVHD in rats (3–7) and milder GVHD in several strains of mice (8). In addition, CSA treatment of neonatal rodents leads to organ-specific autoimmune disease (9, 10). The role of CSA in these models appears to be multifold: i.e., arrest of thymocytopoiesis at the double-positive (DP; $CD4^+8^+$) stage (11–13); interference with positive and negative intrathymic selection (14–16), possibly secondary to selective inhibition of cytokine production (17, 18) and/or damage to stromal cells (19–25); and inhibition of regulatory T cell maturation (13). The net effect of these actions is to skew the T cell repertoire towards unregulated effector T cells bearing self-reactive TCRs. However, little is known about the origin, phenotype(s), and fate of these autoreactive T cells.

It has been widely reported that long-term CSA treatment in mice leads to the accumulation of double-negative ($CD4^-8^-$) T cells in the peripheral lymphoid tissues, some of which may be T cells that have escaped negative intrathymic selection (11, 13, 19). In contrast, we (26, 27) and others (8, 28) have recently reported that short-term CSA treatment of young adult rats results in the appearance in LN of large numbers of double-positive T cells. In addition, we have demonstrated that most of these cells are very small and have

[1] *Abbreviations used in this paper:* CSA, cyclosporin A; DP, double positive; RTE, recent thymic emigrants; SPL, spleen; TdT, terminal deoxynucleotidyl transferase.

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a TCR α/β^{low}, Thy-1$^+$, terminal deoxynucleotidyl transferase–positive (TdT$^+$) phenotype (26, 27). This phenotype and size profile are normally expressed only by cortical thymocytes (29), thereby suggesting that these DP LN T cells are recent emigrants from the thymus cortex.

In the present study, the kinetics of appearance of DP T cells is defined during a short-term course of CSA treatment, and their intrathymic origin is traced by direct intrathymic labeling with FITC. The results demonstrate that >95% of medullary thymocytes and, in half of the animals, ~50% of TdT$^+$ cortical thymocytes emigrate to the peripheral lymphoid tissues during the first week of CSA treatment, after which export of thymocytes ceases. The implications for autoimmunity of the release of a wave of potentially unselected cortical thymocytes to the periphery is discussed.

Materials and Methods

CSA Treatment of Rats. 4–6-wk-old female inbred Lewis rats, bred and housed in our animal care facility, were used in this study. CSA (provided by Sandoz Inc., East Hanover, NJ) was administered, via the oral route, in their food. Powdered CSA was mixed into standard rat chow in lieu of sucrose at a concentration of 0.015 or 0.027% and pelleted (Dyets, Inc., Bethlehem, PA) (30). Rats were maintained ad libitum on either the CSA-containing food, or on the same food lacking CSA, for 1–42 d. Previous studies have demonstrated that this protocol establishes mean serum levels of CSA equivalent to that accomplished by parenteral administration of a therapeutic dose (15 mg/kg body weight/d) (19).

Cells. LN were procured either by biopsy from the cervical and inguinal areas or at necropsy from cervical, axillary, brachial, and inguinal areas. To prevent disruption of the thymus and its cross-contamination of peripheral lymphoid tissues, animals were killed with an overdose of ether (rather than by cervical dislocation) until all cardiac activity ceased, and the thymus was harvested after LN and spleen (SPL). Single-cell suspensions of LN, SPL, and thymus were made by pressing tissues through stainless steel tissue sieves into cold RPMI.

Antibodies. The following mouse mAbs were purchased from Bioproducts for Science (Indianapolis, IN); OX8 (anti-CD8) (31), W3/25 (anti-CD4) (31), R73 (directed against a constant determinant of rat TCR α/β) (32), OX22 (anti–high molecular weight CD45RC) (33), and OX7 (anti-Thy-1.1) (34). The rat mAb DS4.23 (anti-RT6.1) (35) was kindly provided by Dr. D.L. Greiner (Department of Pathology, University of Connecticut Health Center). Most of these mAbs were obtained as either FITC, PE, or biotin conjugates. When the conjugated form was not available, the antibodies were conjugated with FITC or biotin according to the method of Goding (36). Red 613–conjugated streptavidin (tandem conjugate of PE and Texas red) was purchased from GIBCO BRL (Gaithersburg, MD). FITC-conjugated goat anti–rat IgG H + L chains (mouse serum absorbed) and goat anti–mouse IgG H + L chains (rat serum absorbed) were obtained from Caltag Laboratories (San Francisco, CA). Affinity-purified F(ab')$_2$ rabbit IgG antibody to homogeneous calf TdT was obtained from Supertechs, Inc. (Bethesda, MD).

Immunofluorescence. Direct and indirect immunofluorescence assays for surface antigens on lymphoid cells were performed as described (37). Cells were processed on a FACScan® flow cytometer (Becton Dickinson & Co., Mountain View, CA) equipped with an argon ion laser (488 nm); and data from two- to three-color immunofluorescence samples were analyzed using the FACScan® Research Software (Becton Dickinson & Co.). Dead cells, erythrocytes, and myeloid cells were excluded on the basis of forward and side scatter. Immunofluorescent staining for intranuclear TdT was performed as described (27) and examined by fluorescence microscopy.

Intrathymic Labeling of Thymocytes with FITC. Rats were injected intrathymically with FITC (20 μl of 1 mg/ml FITC per lobe) as described (37). Animals were killed at various intervals after the intrathymic injection, and LN, SPL, and thymus were harvested. Single-cell suspensions were made and examined for surface antigens by flow immunocytometry. Sufficient data (2,000–5,000 events) was obtained by live gating to permit detailed analysis of the minor subset of FITC$^+$ cells (0.1–1.0%). Data for total lymphocytes were acquired on the same sample with the FITC gate off to minimize variations in fluorescence staining between recent thymic emigrants (RTE) and total cells.

Results

Kinetics of Appearance of DP T Cells in LN of CSA-treated Rats. Consistent with our previous reports (26, 27), a dramatic increase in the percentage of DP T cells in the peripheral lymphoid tissues occurred in ~50% of CSA-treated rats, whereas the remaining CSA-treated rats showed only a slight increase in DP T cells. When present in large numbers, the DP cells were detected by day 3 of CSA treatment and increased progressively to reach peak levels of up to 70% by day 7 (Fig. 1). This increase in DP T cells was associated with a four- to sixfold decrease in the percentage of DN (CD4$^-$8$^-$) B cells and a two- to threefold decrease in the percentage of SP (CD4$^+$8$^-$ and CD4$^-$8$^+$) T cells. Inasmuch as the absolute number of B cells in SPL remained constant, these results indicated that the total number of T cells was increased by approximately twofold in these rats, primarily by the accumulation of DP T cells.

Further phenotypic analysis (Fig. 2) showed that the small size and TCR α/β^{low}, Thy-1$^{hi/med}$ fluorescence profiles of the DP T cells in LN of CSA-treated rats were almost identical to those of immature cortical thymocytes; and that most of the DP T cells were TdT$^+$ (data not shown). This analysis also showed that CSA treatment resulted in loss of medium size, Thy-1^{low}, TCR α/β^{hi}, TdT$^-$ T cells within 7 d. We have previously shown (37) that such T cells have a single-positive, RT6$^-$, CD45RC$^-$ phenotype characteristic of RTE from the thymus medulla and their immediate descendants.

Additional evidence that the DP T cells that appeared in LN of CSA-treated rats were RTE was found in the inverse correlation between the mean percentage of DP T cells in LN of groups of rats (Fig. 3 *A*) and the mean number of thymocytes in these same animals (Fig. 3 *B*) as a function of time. Indeed, as shown in Table 1, those rats that had <700 × 10^6 (mean, 568 × 10^6) thymocytes 3–7 d after CSA treatment also had a significantly higher mean percentage of DP, Thy-1^{hi}, TCR α/β^{low} LN T cells than did those that had >700 × 10^6 (mean, 947 × 10^6) thymocytes (p <0.001). This correlation suggests that the animals with the smallest thymi were those that had cumulatively exported the largest

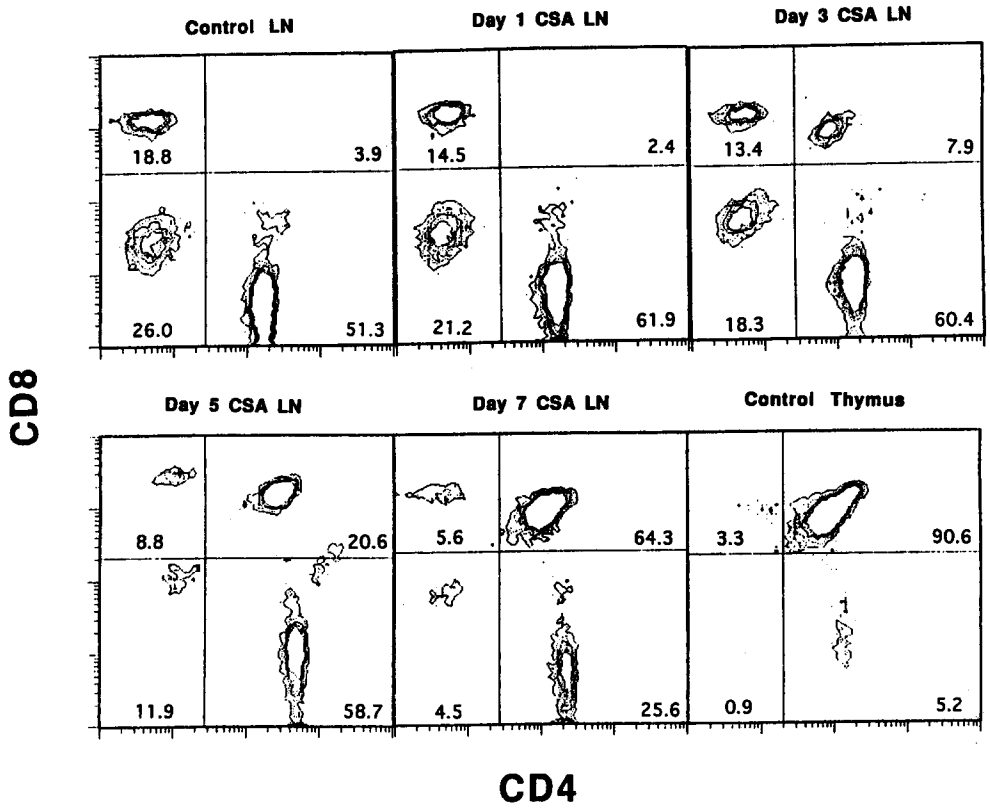

Figure 1. Two-color gated flow cytometric analysis of the expression of CD8 and/or CD4 by LN cells from 0.027% CSA-treated and control rats, as compared with normal thymocytes.

numbers of cortical thymocytes to the periphery during the first week of CSA treatment.

To confirm the role of the thymus in maintaining the pool of Thy-1$^+$ T cells in peripheral lymphoid tissues, LN biopsies were obtained at time intervals from thymectomized rats that had been maintained on CSA or control lab chow for up to 32 d. The results in Table 2 show that thymectomy, but not sham-thymectomy, led to the loss of Thy-1$^+$ T cells from LN within 1 wk, presumably by permitting their maturation to Thy-1$^-$ T cells while preventing their replacement by RTE (37). The results also demonstrate that the treatment of rats with CSA either 10 d after or the same day as

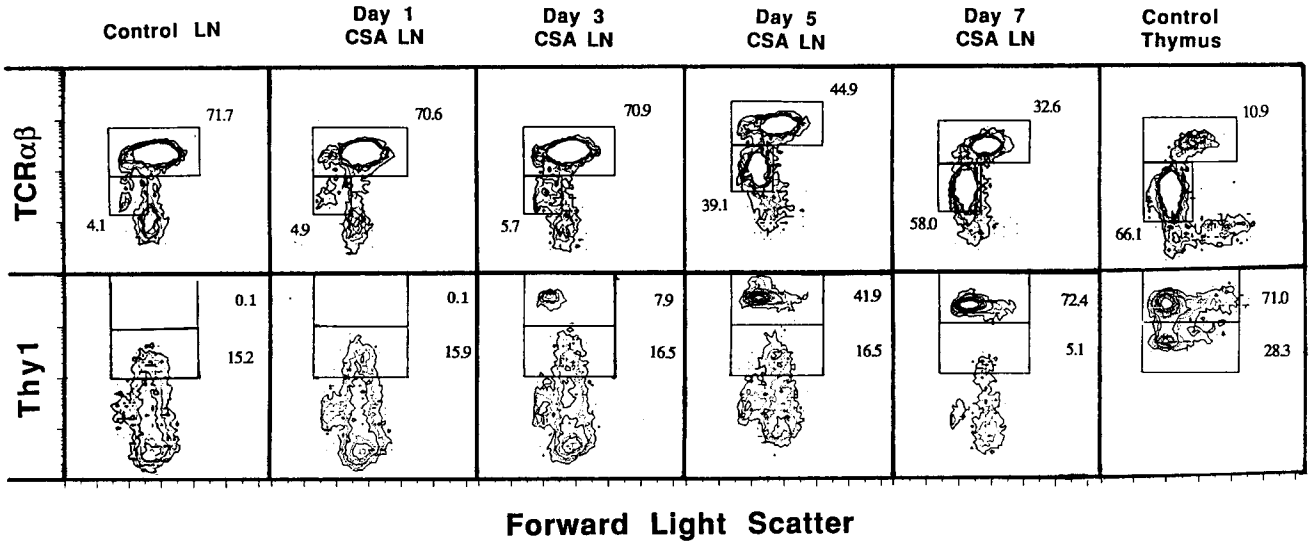

Figure 2. Gated flow cytometric analysis of TCR α/β and Thy-1 expression vs. forward light scatter (relative cell size) by LN cells from 0.027% CSA-treated and control rats. The corresponding profiles of normal thymocytes are included for comparison. The windows demarcate two subsets of TCR α/β^+ cells: the upper window being medium sized, TCR α/β^{hi}; the lower being small, TCR α/β^{low}. The windows also demarcate two subsets of Thy-1$^+$ cells: the upper being mostly small Thy-1$^{hi/med}$; and the lower being medium-sized Thy-1^{low}. The percentage of total LN cells within each window is indicated.

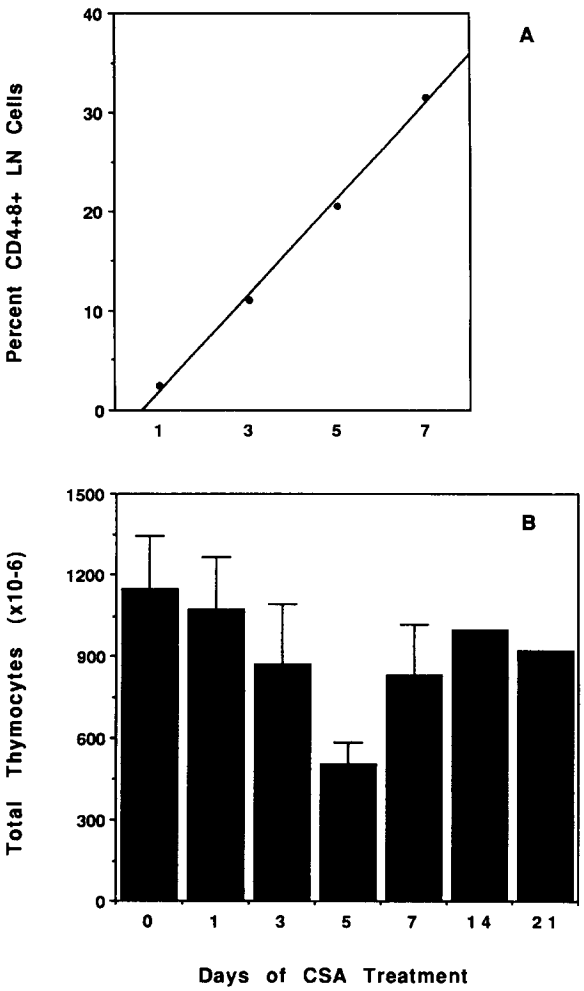

Figure 3. Kinetics of (*A*) increase of DP ($CD4^+8^+$) T cells in LN and (*B*) decrease of total cells in thymus of 0.027% CSA-treated rats. Results in *A* represent mean percent of total LN cells that are DP at the indicated times during CSA treatment (four animals per point), and are presented as the line of best fit for all points as determined by linear regression analysis: $r^2 = 0.99$; slope = 4.85. Results in *B* for days 0–7 depict mean ± SD of total thymocytes from four individual rats, and those for days 14 and 21 represent the averaged values from four pooled thymi.

Table 2. *Effects of Thymectomy and/or CSA Treatment on Thy-1⁺ T Cells in LN*

	Percent of LN T cells expressing Thy-1 after treatment		
Day	Sham-Thymectomy	Thymectomy	Thymectomy + CSA
7	21.6 ± 2.6	1.1 ± 0.3	1.6 ± 0.8
17	22.4 ± 0.6	1.4 ± 0.6	1.7 ± 0.5
32	17.4 ± 0.7	2.9 ± 0.4	3.6 ± 1.1

5–8-wk-old Lewis rats were thymectomized or sham-thymectomized 10 d before initiation of CSA therapy and maintained on a 0.027% CSA or control diet for 32 d. LN cells were obtained through biopsies of cervical or inguinal LN at the indicated intervals, stained with mAbs to TCR α/β and Thy-1.1 antigens, and analyzed by flow cytometry. Results represent mean ± SD of three to six rats.

(data not shown) thymectomy neither prevents the disappearance nor causes the reappearance of Thy-1⁺ T cells in LN. Hence, the qualitative and quantitative effects of CSA treatment on subpopulations of LN T cells appear to result almost exclusively from its effects on the intrathymic development, export, and/or immediate postthymic survival of RTE.

Direct Analysis of RTE in CSA-treated Rats. The intrathymic FITC labeling technique was used to directly document thymic export and to examine the phenotypic characteristics of RTE during CSA treatment. RTE were defined as $FITC^+$ T cells identified in LN 16 h after intrathymic labeling. The results demonstrate that the RTE in CSA-treated rats differed quantitatively and qualitatively from their counterparts in normal rats. Thus, as shown in Fig. 4, the number of cells released from the thymus of these rats per unit time was approximately twofold above control levels by day 3 of CSA treatment, decreased to below control levels by day 5, and ceased entirely by day 9. The changes in the rate of thymic export on each of these days differed significantly ($p < 0.05$) from control values at time 0.

The results in Fig. 5 show that the increase in thymic ex-

Table 1. *Distribution Analysis of the Percentage of $CD4^+8^+$, Thy-1^{hi}, TCR α/β^{low} LN Cells as a Function of the Number of Thymocytes Present in Rats Treated with 0.027% CSA for 3–7 d*

No. of thymocytes		Percent positive LN cells (mean ± SD)		
Range	Mean ± SD	$CD4^+8^+$	Thy-1^{hi}	TCR α/β^{low}
$\times 10^{-6}$				
459–698	568 ± 203	27.6 ± 21.6*	33.5 ± 25.2*	27.8 ± 20.4*
788–1,125	947 ± 140	4.0 ± 3.4	2.14 ± 3.3	5.2 ± 19

* $p < 0.001$.

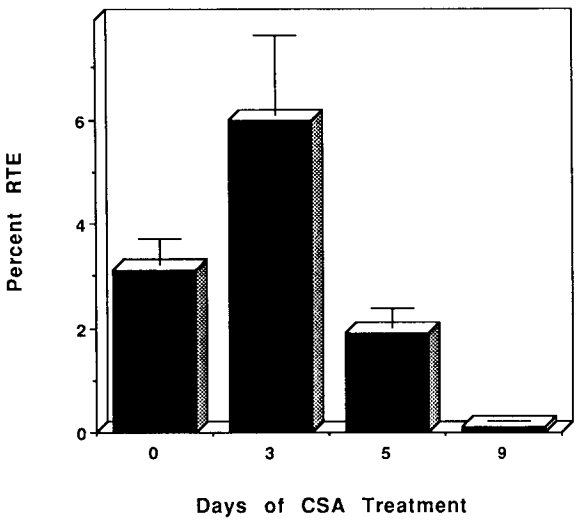

Figure 4. The percentage (mean ± SD) of RTE in LN of normal rats and those treated with 0.015% CSA for 3–9 d. All rats were injected intrathymically with FITC 16 h before death. FITC$^+$ T cells (RTE) were identified by simultaneous flow cytometric analysis for FITC and TCR α/β^+ cells. The proportion of FITC$^+$ LN cells was significantly greater than normal after 3 d of CSA therapy ($p < 0.05$) and significantly lower on days 5 ($p < 0.02$) and 9 ($p < 0.001$).

port in CSA-treated rats was due almost entirely to a five- and eightfold increase in the release of DP and TCR α/β^{low} thymocytes, respectively, the difference between these values presumably being the enhanced export of the CD4$^-$8$^+$ precursors of CD4$^+$8$^+$ cortical thymocytes. Thus, in addition to the continued release of the CD4$^+$8$^-$ and CD4$^-$8$^+$ subsets of Thy-1$^{med/low}$, TCR α/β^{hi} medullary thymocytes through day 5 in CSA-treated animals, there was a threefold increase (4.3 to 11.8%) in the release of the Thy-1^{med} subset of DP, TCR α/β^{low} cortical thymocytes on day 3, and the appearance of two new subsets (CD4$^+$8$^+$ and CD4$^-$8$^+$) of Thy-1^{hi}, TCR α/β^{low} T cells, which were not readily detectable in control rats. Unlike the cells in the Thy-1^{med} subset of DP RTE, the cells in the Thy1^{hi} subsets were TdT$^+$ (data not shown). This clearly indicated that they originated from the thymus cortex (38, 39). In aggregate, the Thy-1^{med} and Thy-1^{hi} subsets of DP, TCR α/β^{low} T cells accounted for ~40% of the total RTE in CSA-treated rats on day 3, whereas such cells accounted for ~5% of total RTE in control rats (37). In the experiment in Fig. 5, export of both of the TdT$^+$ and TdT$^-$ subsets of DP RTE from the thymus cortex decreased markedly between days 3 and 5 of CSA treatment, whereas export of SP RTE from the medulla continued through day 5. However, in other experiments in which individual time points were tested, emigration of cortical (as well as medullary) thymocytes was observed on day 5 and, less frequently, on day 7 of CSA treatment (data not shown).

Discussion

Although the emigration of cortical thymocytes to peripheral lymphoid tissues has long been suggested (38), this is to our knowledge the first direct demonstration of this phenomenon, albeit under unphysiological conditions. More-

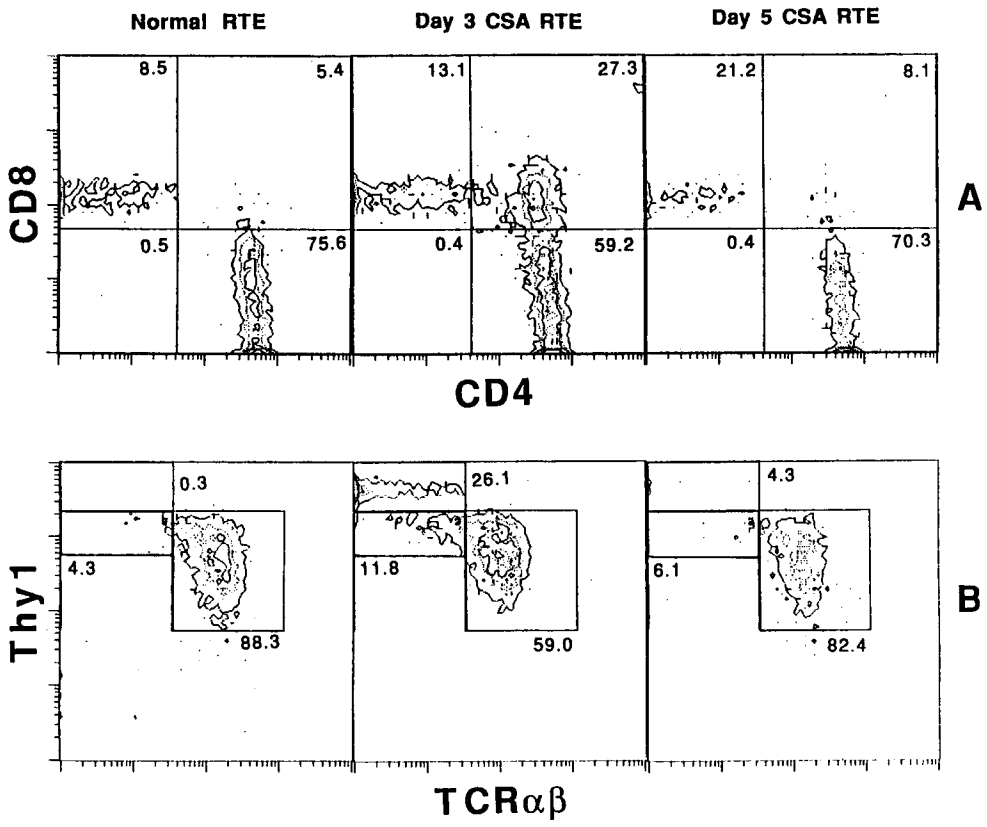

Figure 5. Representative flow cytometric profiles of (*A*) CD4 and/or CD8, and (*B*) TCR α/β and/or Thy-1 expression by RTE in normal rats and those treated with 0.015% CSA for 3 and 5 d. RTE were identified by three-color flow cytometric analysis of LN cells 16 h after intrathymic injection with FITC. The percentage of total FITC$^+$ LN cells (RTE) in each window is indicated.

over, in related papers in preparation, we describe a similar phenomenon in antigen-stimulated rats (Durkin, Seto, and I. Goldschneider, unpublished results), and to a lesser extent in normal rats (37). Nonetheless, despite the qualitative demonstration of cortical thymocyte emigration by phenotypic analysis of intrathymically labeled T cells, some of the quantitative aspects of the present study warrant discussion.

For example, it could be argued that the marked decline in cortical thymocytes in ~50% of CSA-treated rats resulted primarily from increased cell death and/or decreased cell proliferation within the thymus, rather than from increased cell export from the thymus. Several lines of evidence suggest that this is not the case. First, there was an inverse correlation between the size of the thymus and the percentage of DP, TCR α/β^{low}, Thy-1^{hi} T cells present in LN and SPL of individual CSA-treated animals. Second, in those rats in which CSA caused a marked decrease in total thymocytes, DP T cells constituted ~30% of total lymphocytes in LN, thereby increasing the total number of peripheral T cells by an estimated 400×10^6 cells. This calculation assumes a total of 10^9 peripheral T cells in age-, weight- and sex-matched control rats, based on the observation that the SPL contains ~40% of the total T cells in the body (40). Third, the estimated number of immature T cells that appeared in the peripheral lymphoid tissues of CSA-treated rats approximated the total number of cells that were lost from the thymus of the same animals by day 5. Fourth, most of these DP T cells expressed TdT, which normally is present only in immature cortical thymocytes (29, 38, 39). Fifth, in previous studies we have demonstrated that many of these DP T cells express the CS1.21 antigen, which also is specific for a subset of cortical thymocytes in normal rats (26).

These findings are consistent with recent reports from other laboratories of the appearance of DP cells in peripheral lymphoid tissues of CSA-treated, irradiated and bone marrow–reconstituted rats (8, 28). Hence, by as yet undefined mechanisms, CSA appears to induce the release of cortical thymocytes at a less mature stage and in much greater numbers than those released in normal animals. Moreover, after the surge in the release of cortical (and medullary) thymocytes during the first week of CSA therapy, thymic emigration ceases (Figs. 4 and 5), and the number of thymocytes once again increases (Fig. 3 *B*). Kinetic studies of thymocytopoiesis indicate that the halt in thymocyte export during CSA treatment is due to a block in the differentiation of TdT^+ to TdT^- cortical thymocytes, coupled with exhaustion of the supply of preexisting exportable cells from the cortex and medulla (Hossein Zadeh, H., and I. Goldschneider, manuscript in preparation). These studies also show that the increase in total thymocytes during the second week of CSA treatment is due to "piling up" of immature cortical thymocytes behind this block. Most importantly, these studies suggest that the completeness and rapidity of onset of the block in cortical thymocyte differentiation during the first week of CSA treatment are the major determinants of the extent of emigration of cortical thymocytes to the peripheral lymphoid tissues. Thus, it appears that in those animals in which the imposition of the block is rapid and complete, few TdT^+ cortical thymocytes leave the thymus, whereas in those animals in which the block initially is "leaky," large numbers of cortical thymocytes emigrate.

Nonetheless, it still is possible that the variability in the appearance of DP T cells in CSA-treated rats, observed here and in previous studies (26, 27), is due in part to differences in postthymic survival and/or proliferation of RTE in the peripheral lymphoid tissues of individual animals. Indeed, we have recently demonstrated that a large portion of the SP RTE in diabetes-prone, but not diabetes-resistant, BB/W strain rats disappear (apoptosis?) within 24 h of their release from the thymus (Hossein Zadeh, H., D. Greiner, and I. Goldschneider, manuscript in preparation). However, the inverse correlation between the decrease in thymocytes and the increase in RTE after CSA treatment, as well as the arithmetic (as opposed to geometric) accumulation of DP T cells with time, speaks against massive postthymic death and/or selective proliferation of surviving RTE in the present study.

It is also possible that CSA influences the appearance of phenotypically immature T cells by altering peripheral T cell development directly. This does not appear to be true, inasmuch as the present and previous results (37) show that thymectomy selectively causes a decline in Thy-1^+ T cells that is neither prevented nor reversed by subsequent CSA treatment. Thus, our experiments demonstrate that, after permitting (or inducing) a burst of export of cortical thymocytes, CSA induces a delayed pharmacological "thymectomy" by preventing the continued export of any thymocytes. This conclusion is further supported by the report of Beschorner et al. (41), who showed that the elevated proportion of DP T cells in CSA-treated rats decreases markedly between 7 and 21 d after thymectomy and cessation of CSA treatment.

At first glance, it appears that the modest twofold increase in export of RTE in CSA-treated rats observed in Figs. 4 and 5 is insufficient to account for the marked increase in the proportion of DP T cells observed in Figs. 1 and 3 *A*. Therefore, it should be noted that the former figures record the accumulation of RTE over a 16-h period, whereas the latter figures record the accumulation over a period of 7 d. Thus, as we have previously observed in normal rats, although only 3–4% of LN T cells are replaced by Thy-1^+ RTE every 24 h, ~20% of total LN T cells are Thy-1^+, due to the accumulation of differentiating RTE over a 7-d interval (37). Inasmuch as the 16-h export of RTE in CSA-treated rats exceeds that of untreated rats by a mean of 3% of total LN cells (Fig. 4), and inasmuch as most of these extra RTE have cortical thymocyte phenotypes (Fig. 5), an accumulation of ~4.5% RTE above replacement levels every 24 h could readily produce the mean increases in DP LN cells observed in Fig. 3 *A*. Indeed, the linear increment of DP T cells as a function of time in Fig. 3 *A* can itself be best explained by the progressive accumulation of RTE after the onset of CSA treatment. The extent of the increase in DP T cells in individual animals would then depend on the duration of export of DP RTE from thymus and their fate in peripheral lymphoid tissues, both subjects of ongoing investigation.

The functional attributes of the novel subset of DP, Thy-1^{hi}, TCR α/β^{low}, TdT^+ RTE have not been investigated; neither has its long-term fate been determined. However, sev-

eral lines of evidence suggest that many of the cells exported from the thymus of CSA-treated animals may not have undergone or completed intrathymic selection. Thus, positive and negative selection of the TCR α/β T cell repertoire has been reported to involve DP cells that express intermediate levels of TCR (42); and postselection thymocytes are TCR^{hi} (43). In contrast, CSA prevents the maturation of thymocytes to the TCR^{hi} stage (13, 26). Also, CSA interferes with the process of clonal deletion of thymocytes by certain antigens (16, 20); and self-reactive T cells occur in increased frequency in the peripheral lymphoid tissues of CSA-treated animals (14). It is possible that these events are causally related and are the consequence, at least in part, of CSA-induced damage to some of the thymic stromal cells that participate in intrathymic selection, such as medullary epithelial cells and interdigitating dendritic cells (20–25).

Perhaps the strongest evidence against intrathymic selection in CSA-treated rats is our observation that the great majority of the DP RTE express the enzyme TdT, which has been shown to be responsible for the generation of junctional diversity of gene segments in the generation of functional TCR molecules (44). In unpublished studies, we have found that such TdT^+ cortical thymocytes undergo rapid proliferation, whereas most of their TdT^- progeny in the thymus cortex undergo apoptosis. This raises the possibility that the survivors of negative selection constitute a subset of TdT^- cortical thymocytes. Furthermore, the notion that many of the cortical thymocytes that are exported from the thymus during CSA treatment are self-reactive is consistent with reports indicating that the thymus is essential for the induction of syngeneic GVHD and organ-specific autoimmune disease during the first 2 wk of CSA therapy, but not thereafter (4, 9).

Because CSA has been shown to enhance the deletion of superantigen-reactive T cells in the peripheral lymphoid tissues (45), presumably by extrathymic mechanisms of T cell repertoire selection (46, 47), it will be important to determine if CSA also affects the postthymic selection of the TCR repertoire to self-antigens. For if, as we postulate, some of the TdT^+ RTE described in the present study undergo extrathymic maturation without further selection mechanisms, autoaggressive T cells could be generated and, in the absence of effective immunoregulation, permitted to function. If confirmed, this may prove to be one of the mechanisms by which GVHD and organ-specific autoimmune diseases are mediated in CSA-treated animals (3, 9, 43), despite conflicting evidence from other laboratories (8, 48). Moreover, since the $CD45RC^-$ T cell subset appears to play a protective role against GVHD in rats (49, 50), and since most $CD45RC^-$ T cells in normal rats are RTE and their immediate descendants (37), it is possible that the paucity of $CD45RC^-$ T cells in rats treated for >1 wk with CSA (our unpublished observations) may further expedite the development of syngeneic GVHD and other autoreactive disorders in these animals.

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