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Two Developmentally Distinct Populations of Dendritic Cells Inhabit the Adult Mouse Thymus: Demonstration by Differential Importation of Hematogenous Precursors Under Steady State Conditions

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Although a variety of lymphoid and myeloid precursors can generate thymic dendritic cells (DCs) under defined experimental conditions, the developmental origin(s) of DCs in the steady state thymus is unknown. Having previously used selective combinations of normal, parabiotic, and radioablated mice to demonstrate that blood-borne prothymocytes are imported in a gated and competitive manner, we used a similar approach in this study to investigate the importation of the hematogenous precursors of thymic DCs. The results indicate that two developmentally distinct populations of DC precursors normally enter the adult mouse thymus. The first population is indistinguishable from prothymocytes according to the following criteria: 1) inefficient (<20%) exchange between parabiotic partners; 2) gated importation by the thymus; 3) competitive antagonism for intrathymic niches; 4) temporally linked generation of thymocytes and CD8αhigh DCs; and 5) absence from prothymocyte-poor blood samples. The second population differs diagnostically from prothymocytes in each of these properties, and appears to enter the thymus at least partially differently. The resulting population of DCs has a CD8αlow phenotype, and constitutes ~50% of total thymic DCs. The presence of two discrete populations of DCs in the steady state thymus implies functional heterogeneity consistent with evidence implicating lymphoid DCs in the negative selection of effector thymocytes and myeloid DCs in the positive selection of regulatory thymocytes. The Journal of Immunology, 2003, 170: 3514–3521.

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2 Address correspondence and reprint requests to Dr. Irving Goldschneider, Department of Pathology, School of Medicine, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-3105. E-mail address: igoldsch@neuron.uchc.edu
3 Abbreviations used in this paper: DC, dendritic cell; BM, bone marrow; FCM, flow immunocytometric; i.t., intrathymic; PBC, peripheral blood cell.
analysis analogous to that described in fetal thymus organ cultures (24), an essential prerequisite is that the hematogenous precursors of these DCs be indistinguishable from prothymocytes by a number of discriminating criteria. For example, we have recently demonstrated that the importation of prothymocytes is a gated phenomenon in normal adult mice (25), that newly generated prothymocytes are exported from bone marrow (BM) intermittently rather than continuously (D. Foss and I. Goldschneider, manuscript in preparation), and that newly imported prothymocytes compete for a finite number of i.t. binding sites (putative microenvironmental niches) (26). It therefore would be expected that the precursors of at least a subset of thymic DCs shared these properties, and that the generative kinetics of their progeny was linked to that of thymocytes. Conversely, the precursors of non-prothymocytophically derived thymic DCs would be expected to have discernibly different properties. Hence, study of the pattern(s) of importation of DC precursors by the steady state thymus should provide key insights into the developmental origin(s) of the resulting DC population(s).

We approached this objective by using a combination of unmanipulated, parabiotic, and irradiated mice similar to that which we previously used to define the kinetics of prothymocyte importation and thymocytopoiesis in normal adult mice (25–27). The results provided clear evidence for the existence of two developmentally distinct populations of thymic DCs whose precursors are differentially imported from the blood. The first population (~50% of total thymic DCs) appears to arise i.t. from precursors that are indistinguishable from prothymocytes. The second population appears to arise extrathymically from at least partially differentiated precursors, presumably of myeloid origin.

Materials and Methods

Animals

Cohorts of 4- to 6 wk-old female Ly-5 congenic C56BL/6 mice, obtained from the National Cancer Institute, were housed in the Center for Laboratory Animal Care, The University of Connecticut Health Center, until they reached the designated ages. Animals were maintained on commercial mouse chow and water ad libitum.

Preparation of cell suspensions

BM cell suspensions were prepared by flushing the marrow from tibia and femur of 4- to 5-wk-old donor mice with cold RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with sodium bicarbonate (2 mg/ml) and 1% HEPES (1.5 M). Suspensions of nucleated peripheral blood cells (PBCs) were prepared by mixing 0.5 ml of heart blood with 10 ml Alsever’s solution and washing with RPMI 1640. After lysing the RBCs with 0.165 M NH4Cl, the nucleated cells were washed in cold medium and centrifuged at 4°C for 5 min at 1500 rpm. The modified enzymatic digestion technique of Wu et al. (27), using type II collagenase B and grade II dispase (Worthington, Freehold, NJ) and 0.1% DNase (Boehringer Mannheim, Mannheim, Germany), was used to optimize recovery of DCs from thymus lobes stripped of attached lymph nodes. Nucleated cells were counted on a Z1 Coulter Counter (Beckman Coulter, Hialeah, FL).

Intrathymic adoptive transfer assay for prothymocytes

Recipient mice received 6 Gy total body irradiation (0.97 Gy/min) from a 137Cs source (Gamma Cell 40 Irradiator; Atomic Energy of Canada, Orléans, Canada) 2–4 h before BM or PBC injection. After anesthesia (ketamine/acepromazine), the thymus was surgically exposed, and the indicated number of cells were injected into the anterior superior portion of each lobe (10 μl/site) using a 1-ml syringe (with attached 28-gauge needle) mounted on a Tridex Stepper (Indicon, Brookfield Center, CT), as described (28). The skin incision was closed with Nexaband Liquid (Veterinary Products, Phoenix, AZ).

Intravenous adoptive transfer assay for prothymocytes

The indicated number of BM cells suspended in 0.5 ml of RPMI 1640 was injected through a 28-gauge needle into the lateral tail veins of irradiated (6 Gy) unanesthetized recipient mice. Control mice were injected with RPMI 1640 alone.

Flow immunocytometric (FCM) analysis

Thymic cells were harvested at timed intervals after BM cell transfer. The percentages of donor and host origin cells were determined by FCM analysis (FACScan; BD Biosciences, Sunnyvale, CA) after development with anti-Ly-5.1 and anti-Ly-5.2 mAbs (The Jackson Laboratory, Bar Harbor, ME), and the expression of MHCII, CD11b, CD11c, CD8α chain (BD PharMingen, San Diego, CA), and/or NLDC-145 (Serotec, Raleigh, NC) was determined by multicolor analysis using combinations of FITC, PE, Red 670, and APC-labeled Abs. Between 1 × 10⁶ and 2 × 10⁶ viable cells were collected in each file. Specificity and sensitivity of staining were controlled by checkerboard analysis against normal 5.1 and Ly-5.2 thymocytes and purposeful mixtures thereof. The percentage of positive cells was calculated by using the intersection of the fluorescence histogram with its control profile to determine the cutoff points.

In preliminary experiments, >95% of the total MHCII⁺CD11c⁺CD11b⁺ thymic DCs were found among the 1% of thymic cells having the highest forward and side angle light scatter profiles. The DC cells constituted ~0.1% of total thymic cells, were mostly (~70%) NLDC-145⁺, and showed typical DC morphology after sorting. CD3⁺ thymocytes were restricted to and constituted almost all of the cells in the lower light scatter fractions. As reported (29), many of the CD11b⁺ macrophages in the thymus exhibited autofluorescence and therefore also appeared to be CD11c⁺. However, as neither the CD11b⁺ thymic DC nor the thymocytes were autofluorescent, we did not find it necessary to remove or exclude autofluorescent cells before analysis.

Parabiosis

Pairs of 4- to 5-wk-old, Ly-5 congenic mice were surgically joined by cutaneous vascular anastomosis, as described previously (30). Parabiotic mice were maintained for periods of 2–8 wk before sacrifice, or were surgically separated at semiweekly intervals and killed 28 days later. Thymi from four to six pairs of unseparated or separated parabiotic partners were harvested at the indicated time points, and the respective degrees of thymocyte and thymic DC chimerism were determined by FCM analysis.

Results

Effects of thymic involution and bone marrow regeneration on thymocyte and thymic DC precursor activities

If, as postulated, a major subset of thymic DCs is generated i.t. by a common T/DC precursor, the number of DCs per thymus should decrease roughly in parallel with that of thymocytes after the onset of age-dependent thymic involution. Similarly, the generation of the hematogenous precursors of thymocytes and many thymic DCs also should be linked. Although neither prediction would establish a common origin for thymocytes and thymic DCs, failure to satisfy either one would seriously jeopardize this hypothesis.

To test the first prediction, the mean numbers of thymocytes and thymic DCs in a cohort of weanling mice were determined at weekly intervals between 5 and 17 wk of age. The results confirmed that the onset of thymic involution at ~12 wk of age was associated with a progressive decrease of both thymocytes and DCs (data not shown).

To test the second prediction, the kinetics of regeneration of thymocyte and thymic DC precursor activities in BM of sublethally irradiated (6 Gy) mice was determined over a period of 9 wk. As shown in Fig. 1A, the levels of thymocyte and thymic DC chimerism induced by the i.v. injection of regenerating BM cells into irradiated recipients were essentially superimposable at all time points. The results also showed that, at most time points, the numbers of donor and host origin DCs (Fig. 1B), like those of thymocytes (Fig. 1C), were inversely related. Based on our demonstration of competitive antagonism for binding sites between recently imported prothymocytes (26), these latter observations suggest that the precursors of many thymic DCs also compete for a finite number of i.t. niches.
Based on these observations, we reasoned in this study that if a major subset of thymic DCs was derived i.t. from a common T/DC precursor, its hematogenous progenitors should be imported synchronously with prothymocytes. Conversely, the precursors of nonprothymocytically derived DCs would be expected to be imported independently of prothymocytes. As before (25), these predictions were tested in Ly-5 congenic pairs of both unseparated and separated parabiotic mice. In the first group, the kinetics of development of thymocyte and thymic DC chimerism was determined at biweekly intervals over an 8-wk period. In the second group, the parabiotic partners were surgically separated at semi-weekly intervals over a 9-wk period, and the respective levels of chimerism induced by recently imported precursors were determined 28 days later.

The results in the cohort of separated parabionts (Fig. 2) showed almost complete concordance between the kinetics of importation of the precursors of donor origin thymocytes and DCs. This was true even though gate opening was not synchronized between the parabiotic partners (25, 30). Thus, peak levels of thymocyte and DC chimerism (modal points of gate opening) approached 10% and occurred in the Ly-5.1 partners at days 24 and 59–63 of parabiosis, and in the Ly-5.2 partners at days 10, 37–39, and 56–59 of parabiosis. These results indicated that the precursors of thymocytes and a major population of thymic DCs: 1) are both inefficient (~10%) exchanged between parabiotic partners; 2) enter the thymus in a synchronously gated manner; and 3) coordinately generate mature progeny (25, 30). It therefore is likely that the donor origin thymocyte and DC populations in each of the separated parabionts were derived from a common hematogenous precursor.

In contrast, no concordance whatsoever was observed between the kinetics of establishment of peak levels of thymocyte and thymic DC chimerism in the cohort of unseparated parabionts. As shown in Fig. 3, maximal DC chimerism (50–60% donor origin cells) occurred within 2 wk of parabiotic union and plateaued thereafter. Maximal thymocyte chimerism (~20% donor origin cells (30)), in contrast, did not occur until at least 8 wk of parabiosis. As peak thymocyte chimerism occurs ~4 wk after the gated importation of prothymocytes (25), the present results indicate that, unlike prothymocytes, the precursors of this rapidly appearing population of thymic DCs: 1) are randomly exchanged between parabiotic partners; and 2) enter the adult thymus in an ungated fashion. In addition, as most immature lymphohemopoietic cell precursors fail to mix randomly in the blood of parabionts (30–32), the results further suggest that these DC precursors enter the thymus in at least a partially differentiated state. Finally, the fact that this rapidly appearing population of DCs was not readily apparent in the cohort of separated parabionts (Fig. 2) suggests that its i.t. \( t_{1/2} \) is less than 4 wk.

It was important, in reaching these conclusions, to exclude the possibility that the rapid appearance of donor origin thymic DCs relative to thymocytes simply reflected differential generative kinetics from a common i.t. precursor. We therefore compared the kinetics of appearance of DCs and thymocytes in sublethally irradiated mice as a function of time after the i.t. injection of normal BM cells. The results in Fig. 4A show that donor origin DCs and thymocytes were initially detected on days 7 and 10, respectively, and that they reached peak levels on days 21 and 24. This is consistent with other reports of linked thymocyte and thymic DC development (7, 8). Given that the rate of establishment of thymocyte chimerism is similar in irradiated and nonirradiated recipients (25, 28), this 3-day differential in the generative kinetics of thymocytes and thymic DCs was insufficient to explain the 6-wk differential observed in the unseparated parabiotic mice (Fig. 3).
We presume that the population of nonprothymocytically derived thymic DCs did not appear promptly in these recipients because of the immaturity of their precursors in the donor BM (as opposed to blood). Therefore, it is important to note that this population was detected when the regenerative kinetics of host origin thymic DCs was examined. As shown in Fig. 4B, two waves of host origin thymocyte and DC accumulation occurred in the month following sublethal irradiation. In the first wave, peak levels of DCs and thymocytes, presumably arising from a common radiosensitive i.t. precursor (26, 28), were reached at days 7 and 10, respectively. However, in the second wave, peak levels of host origin DCs were reached at least 2 wk before the expected peak levels of thymocytes. As the precursors for the second wave originated in the regenerating host BM (26), these results suggest that the bulk of the early appearing thymic DCs was nonprothymocytically derived.

**Differential generation of thymic DCs by prothymocyte-rich and prothymocyte-poor aliquots of blood**

We have demonstrated in other experiments (E. Donskoy and I. Goldschneider, manuscript in preparation) that, under steady state conditions, the export of thymocyte progenitors from the BM is coordinated with the periodic opening of the i.t. gate for prothymocytes. Hence, at most time points, peripheral blood contains few prothymocytes (28). We therefore determined whether naturally occurring aliquots of prothymocyte-poor as well as prothymocyte-rich blood could generate thymic DCs in adoptive recipients. Toward this end, total leukocytes in 0.5 ml of heart blood from each of 60 normal Ly-5.1 mice, ages 7–12 wk, were injected i.t. into sublethally irradiated 5-wk-old Ly-5.2 recipients (one donor per recipient). The generation of donor origin thymocytes and thymic DCs in each recipient was determined by FACS analysis 24 days later to permit detection of both prothymocytically and nonprothymocytically derived DCs.

The results in Table I show that, although only 17 (28%) of the 60 recipients of normal PBCs developed significant thymocyte chimism (>5% donor origin cells), all displayed substantial thymic DC chimism. However, the mean level of DC chimism was more than twice as great (59 vs 23%; p < 0.01) in the group of mice that exhibited thymocyte chimism (designated THY⁺) than in that which did not (designated THY⁻); and the total number of donor origin DCs was ~3 times as great (34 × 10⁴ vs 12 × 10⁴; p < 0.01). Extrapolations of prior log-dose titrations of i.t. injected BM cells (26, 28) indicated that the THY⁺ mice must have received at least 10-fold more prothymocytes on average than did the THY⁻ mice to achieve the observed bimodal distribution of thymocyte chimism (mean 55 vs 2% donor origin cells; p < 0.01). This is consistent with the observation that the number of host origin thymocytes was significantly lower in the THY⁺ than in the THY⁻ group of mice (Table I), due to competition with donor origin prothymocytes for a finite number of i.t. niches (26). As the number of host origin DC also was lower in the THY⁺ than the THY⁻ group, their precursors also appeared to compete for i.t. niches. Hence, it seems likely that the donor origin thymic DCs in the THY⁻ group arose predominantly from prothymocytic precursors, whereas those in the THY⁺ group arose almost exclusively from nonprothymocytic precursors.

This inference was further supported by the differential staining for the CD8α chain among the thymic DCs in the two groups of mice. As shown in Table II, 81% of the donor origin DCs in the THY⁺ group were CD8⁺, as compared with only 54% of those in the THY⁻ group (p < 0.01). Furthermore, the mean fluorescence intensity of the CD8⁺ DCs in the THY⁺ group exceeded that in the THY⁻ group by 2-fold (p < 0.01; data not shown). Hence, assuming that the mean numbers of CD8⁺ nonprothymocytically derived DCs per thymus were equivalent in both the THY⁺ and THY⁻ groups of mice (~8 × 10⁴), it can be calculated that virtually all of the prothymocytically derived DCs in the THY⁺ group were CD8⁺ and that most were CD8αhigh.
Reciprocal results were obtained with host origin DCs. Thus, as shown in Table II, the proportions, total numbers, and mean fluorescence intensities (data not shown) of the CD8/H11001 host origin DCs in the THY/H11002 group of mice were equivalent to those of their donor origin counterparts in the THY/H11001 group of mice, and the mean fluorescence intensity of the CD8/H11001 host origin DCs in the THY/H11002 group was twice as great as that in the THY/H11001 group (p < 0.01). However, the numbers of CD8/H11002 donor and host origin DCs remained constant in the THY/H11002 and THY/H11001 groups, and the number of CD8/H11002 host origin DCs in the THY/H11001 group matched that of their CD8/H11002 counterparts (p > 0.1). These results suggest that, unlike prothymocytes, the precursors of the nonprothymocytically derived population of thymic DCs (CD8α−/low) do not compete with each other (or prothymocytes) for i.t. niches.

To estimate the proportionate representation of prothymocytically and nonprothymocytically derived DCs in the steady state thymus, we determined the ratio of CD8α+ to CD8α− thymic DCs in a cohort of normal 7- to 12-wk-old mice. A mean of 74 ± 16% of the thymic DCs was found to be CD8α−, and of these approximately two-thirds were CD8α−/high. Hence, assuming that 100% of prothymocytically derived (CD8αhigh) and 50% of the nonprothymocytically derived DCs (CD8α−/low) are CD8−, it can be calculated that both DC populations are equally represented in the young adult mouse thymus.

Discussion

Although a series of recent in vivo studies have indicated that more than one lineage of DCs may populate the mouse thymus under conditions in which thymocytopoiesis, myelopoiesis, and/or the thymic architecture are abnormal (11–13, 15–19), it was not possible to determine whether the observed results were applicable to the normal host. Using normal mice, Kamath et al. (23) recently observed two-phased 5-bromo-2’-deoxyuridine-labeling kinetics consistent with, but not conclusive for, the existence of two types of thymic DCs in normal adult mice. In this study, we document the differential importation of two developmentally discrete populations of DC precursors by the thymus of parabiotic mice. One population is indistinguishable from prothymocytes by the following criteria: 1) parallel kinetics of production in regenerating BM; 2) inefficient (<20%) exchange between parabiotic partners; 3) gated and simultaneous importation by the thymus; 4) competitive antagonism for a finite number of i.t. binding sites; 5) linked generation of thymocytes and thymic DCs; 6) absence from blood samples lacking detectable prothymocytic activity; and 7) parallel onset of age-dependent i.t. involution. Although it theoretically is possible that separate precursors for thymocytes and thymic DCs are simultaneously imported and processed by the thymus, this is
highly unlikely in view of the evidence for a common i.t. T/DC precursor of prothymocytic origin (7–10). Rather, the most parsimonious explanation for the striking similarities between prothymocytes and the hematogenous progenitors of a major population of thymic DCs is that they are identical. Furthermore, the slightly shorter lag period observed for the generation of thymic DCs as compared with thymocytes is consistent with the proposed origin of thymic lymphoid DCs from stage I and stage II triple-negative (CD3<sup>−</sup>, CD4<sup>−</sup>, CD8<sup>−</sup>) thymocytes (8).

The second major population of thymic DC precursors can be readily distinguished from prothymocytes by the following criteria: 1) efficient (random) exchange between parabiotic partners; 2) un gated importation by the thymus; 3) absence of competitive antagonism; 4) un linked generation of thymocytes and thymic DCs; and 5) presence in blood samples lacking detectable prothymocytic activity. Their efficient exchange in the blood of parabionts, their rapid accumulation in the thymus within 1 wk after the establishment of vascular anastomosis (~2 wk after parabiotic union), and their relatively short i.t. lag suggest that these nonprothymocytic precursors are imported as at least partially differentiated DCs. Furthermore, the absence of gating or competition for i.t. niches suggests that they enter the thymus continuously and in numbers re

### Table I. Induction of thymocyte and thymic DC chimerism by PBCs<sup>a</sup>

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>% Donor Origin Cells</th>
<th>No. Donor Origin Cells</th>
<th>No. Host Origin Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>THYs (×10&lt;sup&gt;6&lt;/sup&gt;)</td>
<td>DCs (×10&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>DCs/THY ratio</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>24 ± 28</td>
<td>38 ± 24</td>
<td>2.0</td>
</tr>
<tr>
<td>THY&lt;sup&gt;+&lt;/sup&gt;</td>
<td>17</td>
<td>55 ± 25</td>
<td>59 ± 19</td>
<td>1.1</td>
</tr>
<tr>
<td>THY&lt;sup&gt;−&lt;/sup&gt;</td>
<td>43</td>
<td>2 ± 3</td>
<td>23 ± 10</td>
<td>10.1</td>
</tr>
<tr>
<td>p</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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</table>

<sup>a</sup> Total nucleated PBCs in 0.5 ml heart blood from each of 60 normal Ly-5.1 mice, ages 7–12 wk (groups of 10), were injected i.t. into individual, sublethally irradiated (6 Gy), Ly-5.2 recipients. The level of thymocyte and DC chimerism in each recipient was determined by FACS analysis 24 days after injection. Results represent means ± SD rounded to the nearest whole number.

### Table II. Staining for CD8α on donor and host thymic DCs in chimeras generated by PBCs

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>% CD8α&lt;sup&gt;+&lt;/sup&gt; DCs</th>
<th>No. CD8α&lt;sup&gt;+&lt;/sup&gt; DCs (×10&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>No. CD8α&lt;sup&gt;−&lt;/sup&gt; DCs (×10&lt;sup&gt;3&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Donor origin</td>
<td>Host origin</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>65 ± 25</td>
<td>64 ± 18</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>THY&lt;sup&gt;+&lt;/sup&gt;</td>
<td>17</td>
<td>81 ± 15</td>
<td>51 ± 20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>THY&lt;sup&gt;−&lt;/sup&gt;</td>
<td>43</td>
<td>54 ± 20</td>
<td>68 ± 15</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

<sup>+</sup> Significance of differences of means between THY<sup>+</sup> and THY<sup>−</sup> groups of mice.

<sup>b</sup> Same groups of mice and thymic DCs as in Table I.

<sup>c</sup> Significance of differences of means between heterologous sets of donor origin and host origin cells (indicated by diagonally opposed arrows).

<sup>d</sup> Significance of differences of means between homologous sets of donor origin and host origin cells.
cells have recently been identified in murine lymph nodes and spleen (37). Although our preliminary data indicate that ~10% of CD11b+ CD11c− thymic DCs have a phenotype consistent with that of plasmacytoid DCs (e.g., B220+, CD205−), we have not yet defined their developmental origins or lineage associations.

In addition to the two populations of CD11b− thymic DCs described in this work, it is possible that yet another subset of DCs exists among the population of CD11b+ murine thymic cells, as reported for human thymic DCs (21). Like Vremec et al. (29), we found that the vast majority of the CD11b+ CD11c+ cells resembled macrophages (unpublished observations). Nonetheless, because most CD11b+ cells in the mouse thymus are autofluorescent, it is difficult to determine their precise antigenic phenotypes.

The presence of at least two developmentally discrete populations of DCs in the thymus implies the existence of functional heterogeneity as well. One major function ascribed to i.t. generated lymphoid DCs involves negative selection of potentially autoreactive clones of developing thymocytes (3). The present results suggest that this function is optimized by the gated entry of a common T/DC precursor, which generates parallel waves of thymocytes and lymphoid DCs (see Fig. 5). Not only does this mechanism help to maintain the optimal thymocyte:DC ratio, but it presumably ensures that DCs of the appropriate lineage and stage of development/differentiation/activation are available to interact at the appropriate time and place with developing thymocytes. Such a mechanism theoretically could function under conditions of thymic growth, involution, or regeneration, as well as under steady state conditions. Furthermore, as proposed by Ardavin et al. (7), the synchronization of DC and thymocyte production may provide a mechanism whereby each wave of newly generated thymocytes is selected against a unique panel of i.t. expressed self peptides, thereby periodically updating the TCR repertoire.

The function(s) of the nonprothymocytic population of thymic DCs is more problematic, due not only to its possible heterogeneous origins, but also to possible functional differences between the CD8α+ and CD8α− subsets (38–42). However, several experimental systems suggest that at least some extrathymically generated thymic DCs can efficiently induce the formation, activation, and/or export of immunoregulatory thymocytes. For example: 1) i.t. injected donor MHC II-positive cells (presumably DCs) are responsible for the induction of specific unresponsiveness to cardiac allografts (43); 2) APC-depleted pancreatic islet allografts fail to induce unresponsiveness to donor strain islets when inoculated i.t. (44); and 3) Ag-pulsed thymic DCs protect against the induction of experimental autoimmune encephalomyelitis when transfused into euthymic, but not thymectomized, mice (45). The later study is particularly intriguing, as it suggests that thymic DCs can transfer Ag-specific dominant tolerance to a naive recipient, but only after returning to the thymus.

We have recently observed that i.v. infusion of F4/80+ CD1+ mononuclear cells from the blood of mice injected with Ag in the anterior chamber of the eye rapidly induces the appearance and release of immunoregulatory NKT cells from the thymus of naive recipients (4, 5). Importantly, some of these presumptive APCs, which acquire regulatory properties in the immunosuppressive (immunologically privileged) environment of the eye (46), accumulate as CD11c+ DCs in the thymus of the adoptive recipients (5) (our unpublished observations). It is possible that, under steady state conditions, these regulatory DCs are represented by the minor population of CD8α+ F4/80+ F4/80low F4/80+ CD11c+ thymic DCs (29) or by B220+ plasmacytoid DCs, which have been reported to have tolerogenic potential (1, 47). Chiffoleau et al. (6) have provided yet another example of the ability of presumptive DCs to migrate to the thymus under tolerogenic conditions. In their experiments, donor origin MHCII+ APCs from a cardiac allograft were identified in the thymus of rats treated with the deoxyspergualine analog, L715-0195, and were associated with the expansion of Ag-specific CD4+ CD25+ immunoregulatory thymocytes (48).

In summary, the present study supports the existence of at least two developmentally discrete populations of DCs in the steady state thymus. One population (~50%) appears to be generated i.t. from prothymocytic precursors, and the second extrathymically
from nonprothymocytic precursors. When combined with evidence from the literature and our ongoing studies, these results support a novel paradigm whereby thymus-seeking DCs of nonprothymocytic origin supplement and reinforce the functions of i.t. generated DCs of prothymocytic origin by broadening the repertoire of extrathymic self Ags available for negative selection, and by inducing the i.t. formation, positive selection, activation, and/or export of regulatory T cells on demand.

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