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Regulation of Thymus Size by Competition for Stromal Niches among Early T Cell Progenitors

Susan E. Prockop* and Howard T. Petrie†

Thymic T cell production is characterized by differentiating waves of non-self-renewing, bone marrow-derived progenitors. The factors constraining new progenitor recruitment, intrathymic precursor expansion, and thymus size remain enigmatic, but are believed to be controlled by a feedback loop responding to lymphoid cellularity and competition for stromal niches. In this study, we show that competition for stromal niches does occur, but is solely limited to cells at the early CD4<sup>+</sup>CD8<sup>+</sup> stages of differentiation. The overall size of the organ is determined both by this limitation on early progenitor expansion, and by a second, cell-intrinsic limit on expansion of progenitor cells transiting to the CD4<sup>+</sup>CD8<sup>-</sup> stage. Together with asymmetric use of marrow-derived progenitors to reconstitute the intrathymic pool, these processes facilitate continuous generation of new T cells while maintaining a relatively stable organ size. The Journal of Immunology, 2004, 173: 1604–1611.

The sizes of all organs and tissues are constrained by poorly understood mechanisms (1). In particular, cell production in tissues that undergo steady-state differentiation postnatally must be carefully monitored to maintain health and homeostasis. T lymphocyte production in the postnatal thymus is unique among systems of steady-state differentiation, because self-renewing progenitors do not reside in situ. Rather, new thymic progenitors are derived from precursor cells that circulate in the blood. The rationale for this ectopic delivery system has never been clear. One thing that is clear is that it is not a steady-state process; instead, the thymus alternates between refractory and responsive periods for new progenitor recruitment (2). In turn, this suggests that conditions inside the thymus feed back to vascular/perivascular cells at the sites of new progenitor entry, allowing them to up-regulate recruitment signals as necessary and appropriate. The conditions that induce the thymus to fluctuate between responsive and refractory states also remain unclear, but because there are no self-renewing cells in the thymus, the most intuitive scenario is that as each wave of progenitors is exhausted, a corresponding decrease in thymocyte number would induce the responsive state. However, although the thymus does exhibit a slow and progressive age-associated atrophy after adolescence, no periodic fluctuations in size are apparent. Thus, a feedback loop initiated by a decrease in total thymus cellularity appears unlikely.

Once inside the thymus, each newly recruited progenitor undergoes ~20 relatively symmetric cell divisions (reviewed in Ref. 3) to generate a large pool of immature thymocytes for TCR-mediated selection. Approximately 12 of these cell divisions occur over a 14-day period that spans the CD4/CD8 lineage double-negative (DN)<sup>3</sup> stages of development (3). The remaining proliferation occurs over a 2- to 4-day span that includes the double-positive (DP) stages of differentiation, in particular, the early DP phase (pre-DP or DN4; see Refs. 3–5). Again, the conditions within the thymus that regulate control of proliferation at the DN or DP stages remain unclear.

In this study, we measure the ability of i.v.-transplanted wild-type marrow to proliferate and differentiate in the thymus of different types of immunodeficient or wild-type hosts. We show that thymus size is controlled independently at the DN and DP stages by dramatically different mechanisms. Remarkably, the total cellularity of the thymus does not appear to have any impact on regulation of progenitor recruitment or expansion processes, or to feed back at all in the regulation of thymus size. Instead, expansion at the DN stage is limited by the availability of stromal niches in the thymus, and competition for them by DN cells. At the DP stage, proliferation appears to be intrinsically limited by the DP cells themselves, and does not appear to correlate with the availability of niches, or to any measure of thymic cellularity. Together with finite importation of new progenitors from the blood (2) and their asymmetric commitment to replenish the DN and DP pools (6), these functions cooperate to maintain continuous T cell output while limiting progenitor growth and thymus size.

Materials and Methods

Animals

C57BL/6j (CD45.2) and congenic B6/Ly5.2Cr (CD45.1) mice were purchased from the National Cancer Institute (Frederick, MD). IL-7Rα<sup>–/–</sup> mice (7) (C57BL/6j background) were purchased from The Jackson Laboratory (Bar Harbor, ME), and subsequently maintained by homozygous breeding at Memorial Sloan-Kettering Cancer Center (MSKCC, New York, NY). RAG-2<sup>–/–</sup> mice (8) were originally purchased from Taconic (Germantown, NY), and subsequently bred at the Animal Care Facility of MSKCC. The mice were rested for at least 1 wk after receipt or weaning before transplantation. All mice were housed under pathogen-free conditions in accordance with the procedures outlined in National Institutes of Health Publication No. 86-23. All experiments involving animals were approved by the Institutional Animal Care and Use Committee of MSKCC.

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3 Abbreviations used in this paper: DAPI, 4',6-diamidino-2-phenylindole dihydrochloride; DN, double negative; DP, double positive; EGFP, enhanced GFP; γ<sub>c</sub>, common cytokine receptor γ-chain; SP, single positive.
Cells
Bone marrow was recovered by flushing cells from iliac crests, femurs, and tibiae, followed by filtration through mesh. RBC were lysed using 0.15 M NH₄Cl/1 mM KHCO₃/0.1 mM EDTA. White cells from peripheral blood were prepared using 2% Dextran T500 (Pharmacia Biotech, Piscataway, NJ) and hypotonic red cell lysis as previously described (9). Single-cell suspensions of thymocytes were prepared by gentle dissociation through wire mesh. Hypocellular thymus preparations were prepared using microfuge tubes and microtestes (Brinkmann Instruments, Westbury, NY).

Nonirradiation chimeras
Recipient mice received 2–20 × 10⁶ viable nucleated donor bone marrow cells from isogenic B6L5.2/Cr (CD45.1) mice. Administration was via tail vein injection in 100 μl of PBS. Recipient mice received no prior conditioning. The majority of recipient mice were sacrificed for evaluation of thymic and marrow chimerism 5–8 wk later. Other cohorts were sacrificed up to 5 months after transplant for analysis of long-term reconstitution (data not shown).

Flow cytometric analyses
All cell suspensions were stained and analyzed at 4°C in mouse tonicity HBSS containing 5% FBS and 0.5% DNase (buffer). Samples of cells prepared as described above were washed with buffer and incubated with optimal concentrations of mAbs. Except as indicated, all Abs were prepared, purified, and conjugated on site at MSKCC. Conjugation of Abs to Alexa dyes was performed as recommended by the manufacturer (Molecular Probes, Eugene, OR). Blocking of non-specific binding was performed with purified rat IgG and anti-FcR (clone 2.4G2). Bone marrow and blood were stained with Alexa680-conjugated anti-CD45.1 (clone 100–4), Alexa633-conjugated anti-CD45.2 (clone A-20), PE-conjugated anti-Gr1 (clone RB6-8C5; Caltag Laboratories, Burlingame, CA), and biotin-conjugated anti-B2020 biotin (Caltag Laboratories) followed by streptavidin-conjugated FITC (BD Pharmingen, San Diego, CA). Thymocytes were stained with anti-CD45.1-A680, anti-CD45.2-A633, Alexa488-conjugated anti-c-kit (clone ACK-2), PE-conjugated anti-CD24 (clone GK-1.5), and PE-conjugated anti-CD8 (clone 53-6.7; Caltag Laboratories) for assessment of DP and single-positive (SP) stages. DN populations from chimeric mice were identified by staining with lineage mixture (anti-CD4, -CD8, -CD3, -Gr1, -ID3, -Mac1, -Ter119) followed by Red613-conjugated anti-rat IgG (Caltag Laboratories), then by Alexa488-conjugated anti-CD44, PE-conjugated anti-CD25 (clone PC-61; BD Pharmingen), anti-CD45.1-Alexa680, and anti-CD45.2-Alexa633. DN cells from nonchimeric mice were prepared by depletion of lineage⁺ cells using paramagnetic beads as described in Ref. 4, followed by staining with Alexa488-conjugated anti-c-kit (clone ACK-2), PE-conjugated anti-CD24 (clone M1/69; BD Pharmingen), Alexa633-conjugated anti-CD44, and Alexa660-conjugated anti-CD25. 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI; Molecular Probes) was used at 0.1 μg/ml for dead cell exclusion, and at 10 μg/ml for cell cycle analysis, as described in Ref. 10. Fixation of stained cells for cell cycle analysis was performed as previously described (10). Doublets were eliminated by forward light scatter pulse processing. Samples were acquired on an LSR cytometer (BD Biosciences, San Jose, CA) with modifications as described (10). Postacquisition analysis was performed using FlowJo software (Tree Star, San Carlos, CA). Cell cycle statistics were generated using the Dean-Jett-Fox algorithm in the FlowJo cell cycle platform.

Generation of enhanced GFP (EGFP) transgenic mice
A vector containing EGFP under the control of the chicken β-actin promoter and CMV enhancer (pCX-EGFP) (11) was provided by Dr. A. Nagy (Lumenfeld Research Institute, Toronto, Canada) with the kind permission of Drs. M. Okabe and J.-I. Miyazaki (Osaka University, Osaka, Japan). The promoter/enhancer/coding sequence was injected into C57BL/6 blastocysts and founder mice were generated by the Transgenic Core Facility of MSKCC. Three founders demonstrating germline transmission were used and founder mice were generated by the Transgenic Core Facility of Drs. M. Okabe and J.-I. Miyazaki (Osaka University, Osaka, Japan). The majority of Ab- and donor bone marrow cells were assayed for normal expression of EGFP in all tissues present in the transgenic founder strains to reconstitution by bone marrow transplantation. a. Overall thymus size in IL-7Rα⁺ or RAG-2⁻ mice 4 wk after transplantation with 5 × 10⁶ congenic wild-type bone marrow cells (transpl), or in untreated age-matched controls (untr). IL-7Rα deficiency, but not RAG-2 deficiency, facilitated reconstitution of the thymus to normal size, as indicated by comparison to an age-matched wild-type control (WT). b. Absolute cell counts (mean ± SD) for six to eight mice treated similarly. c. Cells reconstituting the IL-7Rα thymus are of wild-type (congenic) donor origin, while the RAG-2 thymus, which does not increase in size, is composed mainly of endogenous RAG-2-deficient cells. Reconstitution in IL-7Rα-deficient mice is solely a characteristic of the thymus, because the wild-type donor contribution to stem cell chimerism in the marrow occurs at equivalent and predictably low levels in both types of mutant mice (d–f). Identification of lineage-negative bone marrow cells is shown in d, while e illustrates the location of cells defined as stem cells within the lineage-negative population. f. Relative proportions of wild-type donor stem cells in the marrow of nonirradiated IL-7Rα⁻ or RAG-2⁻ mice after transplantation; statistics represent mean ± SD for four recipient animals of each type.
EGFP transgenic mice. After an additional 4 wk, recipient mice were euthanized and their thymuses analyzed after staining with Abs against CD45.1 (Alexa680 conjugate, recognizing the RAG-2-deficient donor) and CD45.2 (Alexa633 conjugate, recognizing the wild-type EGFP transgenic donor, as well as the IL-7Rα−/− recipient). Recipient and wild-type donor cells were discriminated on the basis of EGFP expression. Samples were also stained with anti-CD4-PE (BD Pharmingen) and anti-CD8-biotin (Caltag Laboratories) to also stain with anti-CD4-PE (BD Pharmingen) and anti-CD8-biotin (Caltag Laboratories) and the number of donor cells in the thymus was analyzed 5–6 wk later. Despite being very small, thymuses from RAG-2-deficient mice did not respond to escalating doses of marrow, and remained as refractory to thymic engraftment as wild-type mice. In contrast, thymuses from IL-7Rα−/− deficient mice were readily reconstituted in a dose-dependent manner.

**Results**

Variable responsiveness to thymic reconstitution after marrow transplantation in mice with different thymic immunodeficiencies

To assess whether overall thymic cellularity or differences in progenitor number could affect thymic progenitor homing and expansion, we measured thymic responsiveness to reconstitution by wild-type marrow in various types of nonirradiated immunodeficient mice. Administration of even small doses of bone marrow (5 × 10⁶ unfractionated cells) revealed a range of different responses in such mice. For instance, thymuses from mice lacking the α-chain of the IL-7R (7), which forms part of the heterodimeric receptors for IL-7 and thymic stromal lymphopoietin (12) were stably reconstituted to normal size by such treatment (Fig. 1). The wild-type donor thymocytes in reconstituted mice had normal proportions of all developmental stages (DN1, DN2, DN3, DP, SP, data not shown), suggesting that proliferative expansion was normal. In contrast, other lymphopenic strains, such as mice lacking MLR-2 (RAG-2) (8), developed trace levels of thymic chimerism proportional to that found in marrow stem cells, but did not reconstitute to normal size (Fig. 1). This was not a consequence of a stromal defect in RAG-deficient thymuses, because they can be reconstituted to normal size and structure in radiation chimeras (13).

Similar treatment of other immunodeficient strains (Fig. 2) confirmed that there was a range of differential responses to reconstitution after marrow transplantation. For instance, mice with JAK-3 (14–16) or common cytokine receptor γ-chain (γc) (17–19) deficiencies, both of which exhibit impaired responses to IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (reviewed in Ref. 20), reconstituted in a manner similar to mice lacking IL-7Rα, while mice lacking the TCR β-chain (21) were as refractory as RAG-2-deficient mice. Mice lacking DNA protein kinase (more commonly known as SCID) (22) displayed an intermediate level of reconstitution, as discussed later in the study. In the remainder of experiments shown in this study, IL-7Rα−/− and RAG-2−/− mice were used as models for responsive or refractory strains. Reconstitution seen in responsive (e.g., IL-7Rα) vs refractory (e.g., RAG-2) strains was thymus specific, because marrow chimerism was present at similar levels in all immunodeficient strains except nude.

**Histology and immunofluorescent microscopy**

Tissues were removed after euthanasia and embedded in OCT (Fisher Scientific, Pittsburgh, PA). Transverse cryosections of 5-μm thickness were prepared and fixed in ice-cold acetone. Light microscopy was performed after staining with Harris’ modified hematoxylin (Fisher Scientific). Fluorescence microscopy was performed essentially as described (6), using FITC-conjugated anti-pancytokeratin (clone C11; Sigma-Aldrich, St. Louis, MO) and Alexa594-conjugated anti-CD25. Samples were mounted in Prolong (Molecular Probes) containing DAPI (0.25 μg/ml), and photographed on a fluorescent microscope using mercury illumination. Monochrome blue (DAPI), green (FITC), and red (Alexa594) images were merged and colorized using IPLab software (Scanalytics, Fairfax, VA).
The thymus is composed of cells of hemopoietic origin (mostly immature T lymphocytes) as well as nonhemopoietic stroma (24). Mutations that lead to a deficiency in lymphoid cells, including RAG-2 and IL-7Rα, can also indirectly affect the thymic stroma (25). To determine whether the difference between responsive (e.g., IL-7Rα−/−) and refractory (e.g., RAG-2−/−) strains was primarily a reflection of the hemopoietic components of the thymus, or of other differences in the thymuses of these mice, several types of experiments were performed. In the first of these (Fig. 4), nonirradiated IL-7Rα−/− mice were transplanted with RAG-2-deficient bone marrow 3 wk before transplantation with wild-type marrow. The resulting triple-chimeric mice were then analyzed as described for the experiments shown in Fig. 1. Cells of the three different origins could be identified in chimeric mice because wild-type donor cells were EGFP+ as well as CD45.2+, while IL-7Rα−/− (recipient) cells were CD45.2+ and EGFP−, and RAG-2−/− cells were CD45.1+. Transplantation with RAG-2-deficient marrow transformed the thymus of IL-7Rα-deficient mice from a responsive phenotype (i.e., capable of reconstitution by wild-type cells) to a refractory one. Donor cell chimerism (both RAG-2−/− and wild type) in the marrow of IL-7Rα−/− mice remained low, as is expected for nonirradiated recipients (23), and consistent with the data shown in Fig. 1. Together, these data show that responsiveness of the IL-7Rα−/− thymus to reconstitution by wild-type progenitors can be modulated by the presence of RAG-2-deficient thymocytes, i.e., DN cells, and the effects that they confer on the thymic stromal microenvironment.

Responsive mice and refractory mice differ in their numbers of DN progenitors

The data in Fig. 4 indicate that RAG-2-deficient DN thymocytes are capable of rendering the IL-7Rα-deficient thymus refractory to reconstitution by wild-type cells. To evaluate the mechanism of this, we compared DN cell distributions in these two strains of immunodeficient mice. Consistent with their published phenotype (8), thymuses from RAG-2-deficient mice consisted solely of DN cells (Fig. 5). In contrast, thymocytes from IL-7Rα−/− mice displayed essentially normal CD4 vs CD8 distributions, as previously reviewed (26). Because thymuses from these two strains are virtually the same size (Fig. 1) and contain similar total cell numbers (Table I), this means that thymuses from IL-7Rα−/− mice have only 2–3% as many DN cells as those from RAG-2−/− mice. The

Transplantation with RAG-2-deficient marrow transforms IL-7Rα-deficient thymuses from a responsive to a refractory phenotype

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phenotype of the thymus in mice with IL-7Rα signaling deficiencies is complicated, because despite having apparently normal proportions of DP and SP cells, they appear to mostly lack cells at the CD25<sup>−</sup>44<sup>−</sup>DN3 stage (Fig. 5, and Refs. 27–29). Nonetheless, those cells that do fall in the DN3 region of a CD44 × CD25 plot appear to be bona fide DN3 cells, because they undergo a reduction in proliferative rate similar to that of wild-type cells (Fig. 6). Further, IL-7Rα<sup>−</sup> DN2 cells (CD25<sup>−</sup>44<sup>−</sup>) proliferate at levels indistinguishable from wild-type DN2 (Fig. 6), suggesting that the apparent paucity of DN3 cells is not due to the inability of thymocytes from IL-7Rα-deficient mice to proliferate, but rather to the absence of a survival signal.

The overall reduction in DN cell number in IL-7Rα<sup>−</sup> mice, and the ability of RAG-2<sup>−</sup> thymocytes to render an IL-7Rα<sup>−</sup>-thymus refractory to reconstitution by wild-type cells, suggests that homing of new progenitors to the thymus (and thus, thymic reconstitution) might be influenced by the absolute number of DN cells present. This is consistent with predictions made by previous studies (2), which showed that importation of new progenitors into the thymus is an actively regulated periodic event initiated by a decrease in thymocyte progenitor number. The data in Fig. 5 and published findings of others (27–29) suggest that the most obvious impact of IL-7Rα<sup>−</sup>-signaling deficiency is at the DN2/DN3 transition. However, DN3 cells are physically removed from the site of blood progenitor entry into the thymus (6, 30), and thus, it is not intuitive that they would influence new progenitor recruitment. To more fully evaluate whether other DN subsets were also reduced, absolute DN1, DN2, and DN3 cell numbers in various immunodeficient strains were calculated (Table I). Note that proper identification of DN1 (CD25<sup>−</sup>44<sup>−</sup>) and DN2 stages requires markers in addition to CD44 and CD25. For instance, many nonlymphoid cells express CD44 (31), and because they are nonlymphoid, they are, likewise, predictably negative for markers such as CD3, CD4, CD8, and CD25. A number of surface markers can help to identify the lymphoid progenitor component within the CD44<sup>−</sup>25<sup>−</sup>lin<sup>−</sup> region (i.e., DN1), but CD117 (c-kit) is especially useful, because it allows clear discrimination between DN2 and DN3 cells (32).

In strains that were readily reconstituted by wild-type marrow (e.g., IL-7Rα<sup>−</sup>, common γ-chain (γc)<sup>−</sup>; see Fig. 2), we found that total DN cell numbers were markedly reduced compared with refractory strains (RAG-2<sup>−</sup>, TCRβ<sup>−</sup>, and wild type). Interestingly, SCID mice, which had an intermediate number of DN cells (Table I), displayed an intermediate level of reconstitution by wild-type marrow (Fig. 2). Together, these data would appear to indicate that the overall number of DN cells might be the most important factor regulating expansion of intrathymic progenitors. However, analysis of individual DN subsets (DN1, DN2, and DN3) reveals that IL-7Rα<sup>−</sup>, γc<sup>−</sup>, SCID, and RAG-2<sup>−</sup> mice all have greatly reduced numbers of DN1 cells, yet reconstitute quite differently, suggesting that DN1 number does correlate well with sensitivity to reconstitution. There is some correlation between DN2 cell number and reconstitution capacity (Table I). However, SCID and RAG-2<sup>−</sup> mice have very similar numbers of DN2 cells, yet differ in reconstitution capacity, while TCRβ<sup>−</sup> mice have far more DN2 cells than RAG-2<sup>−</sup>, yet both strains are similarly resistant to reconstitution. By far, the subset that correlates best with reconstitution capacity is DN3; all mice exhibiting DN3 numbers equal to or greater than wild-type mice are resistant to reconstitution by transplanted marrow, while all mice with less than wild-type numbers are proportionally sensitive (i.e., IL-7Rα<sup>−</sup> ≈ γc<sup>−</sup> < SCID). Thus, the triple-chimeric experiments (Fig. 4) indicate that regulation of thymus cell number is clearly a DN cell-related function, while the data in Table I suggest that it probably involves counting the numbers of DN2 and/or DN3 thymocytes (see Discussion).

**TABLE I. Reduction in absolute progenitor cell numbers in immunodeficient mice**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total Thymocytes</th>
<th>Total DN&lt;sup&gt;+&lt;/sup&gt;</th>
<th>DN1&lt;sup&gt;−&lt;/sup&gt;</th>
<th>DN2</th>
<th>DN3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-7Rα&lt;sup&gt;−&lt;/sup&gt;</td>
<td>9,000 ± 1,800</td>
<td>140 ± 50</td>
<td>0.06 ± 0.04</td>
<td>80 ± 20</td>
<td>20 ± 10</td>
</tr>
<tr>
<td>γc&lt;sup&gt;−&lt;/sup&gt;</td>
<td>8,000 ± 3,400</td>
<td>100 ± 40</td>
<td>0.08 ± 0.06</td>
<td>60 ± 20</td>
<td>20 ± 5</td>
</tr>
<tr>
<td>SCID</td>
<td>2,100 ± 280</td>
<td>1,770 ± 260</td>
<td>0.10 ± 0.08</td>
<td>290 ± 60</td>
<td>1,040 ± 300</td>
</tr>
<tr>
<td>RAG-2&lt;sup&gt;−&lt;/sup&gt;-</td>
<td>9,200 ± 900</td>
<td>8,620 ± 710</td>
<td>0.66 ± 0.05</td>
<td>270 ± 60</td>
<td>8,240 ± 620</td>
</tr>
<tr>
<td>TCRβ&lt;sup&gt;−&lt;/sup&gt;-</td>
<td>7,800 ± 360</td>
<td>4,980 ± 110</td>
<td>22 ± 3</td>
<td>470 ± 70</td>
<td>3,250 ± 130</td>
</tr>
<tr>
<td>Wild-type</td>
<td>152,000 ± 28,000</td>
<td>4,640 ± 310</td>
<td>35 ± 2</td>
<td>330 ± 30</td>
<td>2,120 ± 320</td>
</tr>
</tbody>
</table>

<sup>a</sup> Absolute cell number (×10<sup>3</sup>).

<sup>b</sup> Includes all CD3/CD4/CD8-negative cells.

<sup>c</sup> Defined as c-kit<sup>+</sup> as well as CD25<sup>+</sup> 44<sup>−</sup>.

**FIGURE 6.** Skewed DN subset distribution in IL-7Rα-deficient mice does not result from a proliferation defect. The apparent defect in DN3 production in IL-7Rα-deficient mice is not due to impaired cell division, because DN2 cells from IL-7Rα-deficient mice have virtually identical proportions of S/G2/M cells as their wild-type counterparts. Further, reduced cell cycle activity in DN3 cells from IL-7Rα-deficient mice is likewise similar to that seen in wild-type thymocytes, indicating that proliferation and cell cycle are normally controlled. Statistics represent mean ± SD of S/G2/M cells in DN2 or DN3 thymocytes from three different mice of each type.

**Discussion**

The IL-7Rα<sup>−</sup>-mouse has a miniature, but normally structured, thymus

The presence of DP and SP cells in the IL-7Rα<sup>−</sup> thymus (Fig. 5, and Ref. 33) results in the production of a normally compartmentalized thymus, despite the dramatic overall decrease in cell number (Fig. 7). For instance, in contrast to the RAG-2<sup>−</sup> thymus, defined regions of densely packed cortex and less dense medulla are clearly obvious. The cortex is densely permeated by a reticular matrix of cytokeratin<sup>+</sup> stromal cells, while the medulla is defined
by cytokeratin⁺ cells that are less frequent and stellate in morphology. In contrast, the RAG-2-deficient thymus lacks large regions defined by medullary stromal cells and low cell density, as previously described (34). CD25⁺ progenitors (i.e., DN2 and DN3 cells) are found scattered at low density throughout the cortex of IL-7Rα⁻/⁻ thymuses, while CD25⁺ cells are virtually ubiquitous in the RAG-2-deficient thymus, consistent with the results of flow cytometric staining (Fig. 5). Thus, the IL-7Rα-deficient thymus recapitulates the structure and composition of a normal thymus, except that it is atrophic, deficient in early progenitors, and responsive to reconstitution by normal progenitors. These characteristics indicate that it may be a useful model for studying secondary immunodeficiencies associated with aging and/or viral or chemical agents, as well as for studies of normal blood progenitor homing and thymic reconstitution, as discussed in the next section.

Discussion

The mechanism by which the size of an organ or tissue is regulated remains an open question (1). This is especially true for organs like the thymus, which continue to produce new cells postnatally. The most intuitive mechanism for regulating thymus size would be to continually assess overall cellularity, and to initiate new progenitor recruitment or expansion, as necessary, to maintain organ size. Several lines of evidence presented in this study indicate that this is not the case, and that rather than monitoring overall size, it is the size of the DN pool that is critical. For instance, in nonirradiated RAG-2-deficient mice, transplanted wild-type progenitors reconstitute the thymus to a trace level, consistent with the amount of stem cell chimerism found in bone marrow. In contrast, the thymus of an IL-7Rα-deficient mouse is restored to normal cellularity by the same treatment (Fig. 1). The thymus of an IL-7Rα⁻/⁻ mouse is virtually the same in overall size and cellularity as a RAG-2-deficient thymus (Table I). However, RAG-2-deficient thymocytes consist solely of DN cells, while IL-7Rα⁻/⁻ thymocytes display roughly normal proportions of DN, DP, and SP cells (Figs. 4 and 5, and reviewed in Ref. 26). Thus, the IL-7Rα⁻/⁻ thymus has only 2–3% as many DN cells as that of RAG-2-deficient or wild-type mice (Table I).

Our demonstration that administration of RAG-2⁻/⁻ marrow to IL-7Rα⁻/⁻ mice converts them to a RAG-2⁻/⁻ phenotype (Fig. 4) confirms that the critical feedback parameter involves the number of DN cells, because these are essentially the sole thymic cell type produced by RAG-deficient marrow (Fig. 4). More specifically, these data, which are consistent with the findings of others (32, 35, 36), suggest that of all the DN stages, DN3 cells appear to most strongly factor into the equation, with a possible secondary contribution by DN2. DN1 cells appear unlikely to be involved, because IL-7Rα⁻/⁻, RAG-2⁻/⁻, SCID, and γc⁻/⁻ mice all have reduced numbers of DN1 cells (Table I, and Refs. 32, and 35–37), but differ substantially in their abilities to be reconstituted by wild-type marrow (Fig. 1). DN2 cell numbers correlate somewhat with reconstitution capacity, with IL-7Rα⁻/⁻ and γc⁻/⁻ mice having the fewest DN2 cells and the greatest sensitivity to reconstitution. However, SCID mice actually have slightly more DN2 cells than RAG-2⁻/⁻ mice, yet reconstitute substantially better. The subset that correlates best with sensitivity to reconstitution is DN3; all strains with normal (or supernormal) numbers of DN3 cells are refractory to reconstitution, while strains with subnormal DN3 numbers are reconstituted proportionally to their number (Table I and Fig. 2). Thus, our data indicate that thymus size is controlled by counting DN cell numbers, specifically at the DN3 and, potentially, the DN2 stages.

The ability of DN2/DN3 progenitors to regulate reconstitution in the thymus is consistent with feedback inhibition resulting from competition for occupancy of limited stromal niches. This is noteworthy because we have previously shown that DN2 and DN3 cells (but not DN1) remain constantly in contact with VCAM-1⁺ stromal cells in the cortex (38). Competition for stromal niches is
a widely accepted concept in the hemopoietic system, and has also been shown to play a role in the recruitment of new progenitors to the thymus (2). However, regulation of new progenitor recruitment seems unlikely to contribute to the difference between responsive and refractory strains as defined in this study, because stem cell chimerism in bone marrow was present at virtually identical levels in both categories of mice (see Fig. 1). Further, DN1 cells are, phenotypically and anatomically, the most closely related to thymic homing progenitors from marrow (6, 30, 39), and would appear to be the most likely candidates to feed back to on the progenitor recruitment process. However, as mentioned above, both responsive and refractory strains exhibited a reduction in DN1 cell numbers (Table I, and Refs. 32, and 35–37). Thus, while new progenitor homing to the thymus is almost certainly controlled by competition for specific stromal niches (2), our data suggest that once inside the thymus, competition for intrathymic stromal niches acts independently to regulate the intrathymic expansion process. Thus, there appear to be at least two phases of postnatal thymic lymphopoiesis that are independently impacted by competition for stromal niches (i.e., new progenitor recruitment, and expansion at the DN stages of differentiation).

In contrast to DN cells, which compete for occupancy of a limited number of thymic stromal niches, our data indicate that DP cells, which represent the bulk of thymocytes, do not enter into the equation by which thymus size is constrained. For instance, wild-type cells that do enter the thymus in RAG-2−/− deficient hosts generate normal proportions of DN, DP, and SP cells (Fig. 4, and additional data not shown), but fail to restore the RAG-2−/− genotype by which thymus size is constrained. For instance, wild-type cells that do enter the thymus in RAG-2−/− deficient hosts generate normal proportions of DN, DP, and SP cells (Fig. 4, and additional data not shown), but fail to restore the RAG-2−/− thymus to normal size. This indicates that proliferative expansion at the DP stage must be intrinsically limited; otherwise, any wild-type cells that gained access to the RAG-2−/− thymus should fully reconstitute the DP and SP compartments, where there is no competition by RAG-2−/− cells. This conclusion is completely consistent with a previous report by Almeida et al. (40), who showed that the number of DP cells in the thymuses of competitive (radiated) mice are readily reconstituted in this study, because stem cell chimerism in bone marrow was present at virtually identical levels in both categories of mice (see Fig. 1). Further, DN1 cells are, phenotypically and anatomically, the most closely related to thymic homing progenitors from marrow (6, 30, 39), and would appear to be the most likely candidates to feed back to on the progenitor recruitment process. However, as mentioned above, both responsive and refractory strains exhibited a reduction in DN1 cell numbers (Table I, and Refs. 32, and 35–37). Thus, while new progenitor homing to the thymus is almost certainly controlled by competition for specific stromal niches (2), our data suggest that once inside the thymus, competition for intrathymic stromal niches acts independently to regulate the intrathymic expansion process. Thus, there appear to be at least two phases of postnatal thymic lymphopoiesis that are independently impacted by competition for stromal niches (i.e., new progenitor recruitment, and expansion at the DN stages of differentiation).

The remarkable susceptibility of IL-7Rα−/− mice, and other mutant strains with DN deficiencies, to thymic reconstitution by wild-type marrow has a number of implications. An obvious one is in regard to the characterization of the elusive nature of intrathymic stromal niches, and the signals they deliver to developing lymphocytes. Another is in regard to the plasticity of thymic stroma and its response to engraftment by lymphoid progenitors, including the modulation of signals that occur in response to occupancy or vacancy of stromal niches. The current model also has the advantage of facilitating analysis in untreated recipients, because it does not require high level marrow chimerism, and consequently, the administration of treatments (drugs or radiation) that may irreversibly damage thymic stroma are not necessary. Irradiation is generally required to induce marrow “space”, and our data using nonirradiated chimeras are consistent with this requirement to generate high-level chimerism (Fig. 1). However, despite generation of only trace levels of stem cell chimerism in marrow (Fig. 1), the thymuses of nonirradiated IL-7Rα−/− mice are readily reconstituted by those few wild-type progenitors that are present, due to their characteristic low numbers of DN cells (Figs. 3 and 5). This raises the potential for a second, less obvious application, which is the use of this model to evaluate various T cell immunodeficiencies, especially secondary immunodeficiencies disorders resulting from chemical or radiation damage, or age. For instance, therapeutic conditioning before bone marrow transplantation results in prolonged T cell lymphopenia, particularly in adults (41, 42). The basis for this is unclear, but it appears to be an intrinsic characteristic of the thymic stroma (43). Because our data show that the IL-7Rα−/− mouse thymus is exquisitely responsive to engraftment by transplanted progenitors, it may make an excellent model for assessing the detrimental effects of various conditioning regimens in the adult thymus, and for the aging thymus in general.

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References
gogenous progenitors by the thymus is a gated phenomenon in normal adult mice. J. Exp. Med. 193:365.
etiation and mapping of intrathymic-signaling environments by stem cell trans-


