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Homeostasis Limits the Development of Mature CD8+ But Not CD4+ Thymocytes

Joost P. M. van Meerwijk,1 Samuel Marguerat,2 and H. Robson MacDonald3

The involvement of a variety of clonal selection processes during the development of T lymphocytes in the thymus has been well established. Less information, however, is available on how homeostatic mechanisms may regulate the generation and maturation of thymocytes. To investigate this question, mixed radiation bone marrow chimeras were established in which wild-type T cell precursors capable of full maturation were diluted with precursors deficient in maturation potential because of targeted mutations of the RAG1 or TCR-α genes. In chimeras in which the majority of thymocytes are blocked at the CD4−CD8−CD25+ stage (RAG1 deficient), and only a small proportion of T cell precursors are of wild-type origin, we observed no difference in the maturation of wild-type CD4−CD8−CD25+ cells to the CD4+CD8+ stage as compared with control chimeras. Therefore, the number of cell divisions occurring during this transition is fixed and not subject to homeostatic regulation. In contrast, in mixed chimeras in which the majority of thymocytes are blocked at the CD4+CD8+ stage (TCR-α deficient), an increased efficiency of development of wild-type mature CD8+ cells was observed. Surprisingly, the rate of generation of mature CD4+ thymocytes was not affected in these chimeras. Thus, the number of selectable CD8 lineage thymocytes apparently saturates the selection mechanism in normal mice while the development of CD4 lineage cells seems to be limited only by the expression of a suitable TCR. These data may open the way to the identification of homeostatic mechanisms regulating thymic output and CD4/CD8 lineage commitment, and the development of means to modulate it. The Journal of Immunology, 1998, 160: 2730–2734.

During the maturation of the earliest detectable thymic precursor cells into fully mature thymocytes several clonal selection processes are known to limit the final output of the thymus. CD4+CD8− cells will only mature to the CD4+CD8± stage if a functional pre-TCR complex, comprised of αβ and TCR-β, is expressed at the cell surface (1). This process is known as β selection and increases the proportion of useful precursors that will subsequently rearrange the TCR-α gene. Signaling through the pre-TCR complex is known to induce these maturation events although a ligand (if it exists) for the pre-TCR remains to be identified (2). In recombinase-activating gene I-deficient (RAG1−/−) mice CD4−CD8−CD25+ thymocytes do not differentiate to the CD4+CD8+ stage as a consequence of inability to express a pre-TCR (3). Once CD4+CD8+ thymocytes express the clonotypic TCR-αβ complex they undergo clonal selection processes that result in a T cell repertoire capable of recognition of self-MHC molecules (“positive selection”) while depleted of autoreactive cells (“negative selection”) (4–6). Thymocytes recognizing suitable MHC-peptide ligands will undergo differentiation events characterized by the down-modulation of one of the two coreceptors, up-regulation of the TCR complex, and modulation of the expression of activation and maturation markers (7–10). Fully mature T lymphocytes (characterized by the expression of either CD4 or CD8, high levels of the TCR and Qa-2, and low levels of HSA) (11, 12) leave the thymus to populate the peripheral lymphoid organs. Immature CD4+CD8− thymocytes do not progress to the mature CD4+ or CD8+ stage in the absence of TCR-α, e.g., in TCR-α-deficient (TCRα−) mice (13).

Several reports have established that only around 3 to 5% of thymocytes fully mature (14, 15). Part of this relatively low efficiency results from the limited number of immature thymocytes with appropriate specificities. Thus, estimates of the proportion of MHC-reactive, preselection CD4+CD8+ thymocytes are in the range of 5% (16). Moreover, in mice lacking thymic negative selection, the output of mature T cells is increased approximately twofold (17, 18). Even in TCR-transgenic mice in which practically all thymocytes express selectable TCRs, not all of the thymic precursors will completely mature. The availability of a limited number of “selecting niches” in the thymus has been suggested to be responsible for this observation (14). Finally, limiting the availability of MHC class II- or class I-expressing thymic stromal cells in vitro has been shown to cause a reduction in the development of mature CD4+ and CD8+ cells, respectively (19). Thus, both TCR specificity-related and unrelated mechanisms may limit the thymic output.

To investigate in vivo whether the rate of thymic precursor cell generation and of positive selection is subject to homeostatic regulation in mice with a normal immature TCR repertoire, we studied the development of immature and mature thymic populations in mixed hemopoietic chimeras containing limited numbers (approximately 10%) of selectable wild-type precursors diluted in mutant precursors unable to mature to the immature CD4−CD8+ (RAG1−) or mature CD4+ or CD8+ (TCR-α−) stage. In all cases selectable precursors were derived from B6.PL mice, so that TCR...
specificity-determined clonotypic competition (20) would not influence the results. If homeostatic mechanisms exist that limit progression through a particular developmental stage, the 10-fold reduction in normal precursors capable of maturation should allow a larger fraction of those cells to proceed. Our results indicate that the development of mature CD8\(^+\) (but, interestingly, not mature CD4\(^+\)) and immature CD4\(^+\)CD8\(^-\) thymocytes is subject to homeostatic regulation.

### Material and Methods

#### Mice

C57BL/6 mice were obtained from Harlan (Zeist, the Netherlands). C57BL/6 mice deficient in the expression of the genes encoding TCR-\(\alpha\) (13) and RAG1 (3), as well as C57BL/6 congenic mice expressing the Thy1.1 allele (B6.PL), were purchased from The Jackson Laboratory (Bar Harbor, ME).

#### Bone marrow chimeras

Lethally irradiated (1000 rad \(\gamma\)-irradiation, \(^{137}\)Cs source) C57BL/6 hosts were reconstituted by i.v. injection of 1 to 2 \(\times\) 10\(^6\) bone marrow cells depleted of T cells using anti-Thy1.2 (ATK3) (21) and anti-Thy.1 (HO-22.1.1) (22) Ab and complement (Saxon Europe Ltd, Suffolk, U.K.). The chimeras were kept on antibiotic-containing water (0.2% Bactrim, Roche, Basel, Switzerland) for the duration of the experiment (usually 6 wk).

#### Abs

Flow cytometric analyses were performed using the following abs: anti-Thy-1.2-FITC (AT15) (21); anti-TCR-\(\beta\)-FITC (H57-597, PharMingen, San Diego, CA); anti-H-2K\(^+\)-FITC (AF6-88.5, PharMingen); anti-TCR-\(\beta\)-PE (H57-597, PharMingen); anti-CD25-PE (PC61, Caltag, San Francisco, CA); anti-CD4-PE (H129.19, Boehringer Mannheim, Mannheim, Germany); anti-Thy-1.2-biotin (AT15, revealed with streptavidin-PE, Caltag); anti-CD4-Red613 (H129.19, Life Technologies, Gaithersburg, MD); anti-CD8-Red613 (53-6.7, Life Technologies); anti-CD25-Red613 (3C7, Life Technologies); anti-CD4-APC (RM4-5, PharMingen); and anti-CD8-APC (53-6.7, PharMingen). Analyses were performed using FACSscan and FAccSort\(^{\text{TM}}\) flow cytometers (Becton Dickinson, San Jose, CA).

#### Postirradiation thymic reconstitution

Hemopoietic chimeras were sublethally irradiated (700 rad gamma irradiation, \(^{137}\)Cs source) 6 wk after engraftment. Animals were analyzed 9 to 13 days later. Mature thymocytes were CD4\(^+\)CD8\(^-\)TCR\(^{\text{low}}\) or CD4\(^-\)CD8\(^+\)TCR\(^{\text{high}}\). The fraction of these cells that were of B6.PL (Thy1.1) origin was determined by Thy-1.2 staining. Four-color experiments in which the mature, B6.PL-derived cells were identified as CD4\(^+\)CD8\(^-\)H-2K\(^{\text{b}}\)-lowThy-1.2\(^{-}\) and CD4\(^-\)CD8\(^+\)H-2K\(^{\text{a}}\)-highThy-1.2\(^{-}\) yielded similar results.

### Results

#### Homeostatic mechanisms do not limit development of CD4\(^+\)CD8\(^-\) precursors to the CD4\(^-\)CD8\(^+\) stage

Lethally irradiated C57BL/6 hosts were reconstituted with a mixture of B6.PL and RAG1\(^{-}\); or C57BL/6-derived bone marrow ((RAG1\(^+\) + B6.PL) \(\rightarrow\) B6 and (B6 + B6.PL) \(\rightarrow\) B6 chimeras). The number and proportion of injected B6.PL-derived bone marrow cells was kept constant (10%) and the expected degree of chimerism (10–20%) was observed among CD4\(^+\)CD8\(^-\)CD25\(^+\) thymocytes (Table I). We investigated whether the reduced number of total selectable precursors in (RAG1\(^+\) + B6.PL) \(\rightarrow\) B6 chimeras (Fig. 1, A and B, Table I) allows those cells to compensate by increasing the degree of cellular expansion taking place during the CD4\(^+\)CD8\(^-\)CD25\(^+\) to CD4\(^-\)CD8\(^+\) transition. The ratio of the absolute number of B6.PL-derived (Thy1.1) CD4\(^+\)CD8\(^-\)TCR\(^{\text{low}}\) to CD4\(^-\)CD8\(^+\)CD25\(^+\) thymocytes was used as an indication of the degree of expansion occurring between these two stages. Sublethal irradiation causes most thymocytes to die, followed by the expansion and synchronized differentiation of a surviving precursor population (23). To avoid measuring long-term accumulations, we sublethally irradiated chimeras and analyzed them 10 to 13 days later. As shown in Fig. 2A, no difference in the ratio of the number of B6.PL-derived (Thy1.1) CD4\(^+\)CD8\(^-\)TCR\(^{\text{low}}\) to CD4\(^-\)CD8\(^+\)CD25\(^+\) thymocytes was observed in the two types of chimeras. This lack of a compensatory increase in cellular expansion is accompanied by the significantly lower number of total thymocytes observed in (RAG1\(^+\) + B6.PL) \(\rightarrow\) B6 than in (B6 + B6.PL) \(\rightarrow\) B6 chimeras (Fig. 2B). Thus, the rate of proliferation occurring during the CD4\(^+\)CD8\(^-\)CD25\(^+\) to CD4\(^-\)CD8\(^+\) transition is apparently not limited by homeostatic mechanisms.

Mature T lymphocytes have been shown to induce differentiation of CD4\(^-\)CD8\(^+\)SCID thymocytes to the CD4\(^+\)CD8\(^-\) stage (24, 25). However, in (RAG1\(^+\) + B6.PL) \(\rightarrow\) B6 chimeras, in which practically normal numbers of B6.PL-derived mature thymocytes are present (Fig. 1C, Table I), no significant numbers of CD4\(^-\)CD8\(^+\) RAG1\(^+\) thymocytes were observed (Fig. 1E, Table I). As expected, C57BL/6- and B6.PL-derived precursors develop into CD4\(^-\)CD8\(^+\) thymocytes in both types of chimeras (Fig. 1, C, D, and F).

#### Development of mature CD8\(^+\) but not CD4\(^-\) thymocytes is limited by a homeostatic mechanism

The next stage in the differentiation of thymocytes, the development of CD4\(^+\) or CD8\(^+\) mature thymocytes from CD4\(^-\)CD8\(^-\) precursors, requires the expression of the TCR-\(\alpha\)\(\beta\) heterodimer. The involvement of homeostatic mechanisms in thymic-positive selection was similarly assessed using hemopoietic chimeras in which a minority (10–15%, Table I) of the CD4\(^-\)CD8\(^-\) precursors expressed the TCR-\(\alpha\)\(\beta\) heterodimer and were therefore potentially selectable (B6.PL, Thy-1.1). The remaining (Thy-1.2) CD4\(^-\)CD8\(^+\) thymocytes did not express TCR-\(\alpha\) protein because of a targeted mutation at the TCR \(\alpha\) locus (13). As expected, the

### Table I. Summary of mixed bone marrow chimeras

<table>
<thead>
<tr>
<th>Chimera</th>
<th>Experiment</th>
<th>Figure(^a)</th>
<th>No.(^b)</th>
<th>Chimerism (% B6.PL)(^d)</th>
<th>Thy-1.2(^+) ((\times)10(^{-6}))(^d)</th>
<th>Thy-1.2(^+) ((\times)10(^{-6}))(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(RAG1(^+) + B6.PL) (\rightarrow) B6</td>
<td>Steady state</td>
<td>1</td>
<td>3</td>
<td>16.6 (\pm) 0.7</td>
<td>1.6 (\pm) 0.7</td>
<td>56.8 (\pm) 26.9</td>
</tr>
<tr>
<td>B6 (RAG1(^+) + B6.PL) (\rightarrow) B6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(B6 + B6.PL) (\rightarrow) B6</td>
<td>Steady state</td>
<td>2</td>
<td>14</td>
<td>17.7 (\pm) 10.7</td>
<td>0.2 (\pm) 0.1</td>
<td>169 (\pm) 6.8</td>
</tr>
<tr>
<td>(TCR-(\alpha)(^+) + B6.PL) (\rightarrow) B6</td>
<td>Steady state</td>
<td>2</td>
<td>12</td>
<td>13.5 (\pm) 3.4</td>
<td>100.9 (\pm) 6.4</td>
<td>30.8 (\pm) 1.3</td>
</tr>
<tr>
<td>(B6 + B6.PL) (\rightarrow) B6</td>
<td>Steady state</td>
<td>2</td>
<td>3</td>
<td>17.2 (\pm) 14.0</td>
<td>37.4 (\pm) 14.0</td>
<td>7.6 (\pm) 5.4</td>
</tr>
</tbody>
</table>

\(^a\) Chimeras were used in experiment shown in indicated figure.

\(^b\) Total number of chimeras analyzed.

\(^c\) Degree of chimerism was determined as percentage of B6.PL-derived (Thy-1.2\(^+\)) thymocytes in the CD4\(^+\)CD8\(^-\)CD25\(^+\) population for the (RAG1\(^+\) + B6.PL) \(\rightarrow\) B6 and control chimeras, and in the CD4\(^-\)CD8\(^+\)TCR\(^{\text{low}}\) population for the (TCR-\(\alpha\)\(^+\) + B6.PL) \(\rightarrow\) B6 and control mice (mean \(\pm\) SD).

\(^d\) Indicated are total numbers of Thy-1.2\(^+\) or Thy-1.2\(^-\) thymocytes (mean \(\pm\) SD).
number and proportion of B6.PL-derived CD4⁺CD8⁺ thymocytes were comparable in the (TCR-α⁺ + B6.PL) → B6 and (B6 + B6.PL) → B6 chimeras (Fig. 3, A and B, Table I). Moreover, while a normal proportion of C57BL/6-derived thymocytes in (B6 + B6.PL) → B6 chimeras fully matured, no mature TCR-α⁺-deficient thymocytes were seen (data not shown).

Although the number of B6.PL-derived CD4⁺CD8⁺ precursors was comparable, 1.5-fold more mature CD8⁺ thymocytes were observed in (TCR-α⁺ + B6.PL) → B6 chimeras than in (B6 + B6.PL) → B6 mice (Fig. 3, A and B, Table I). In contrast, the steady state number of mature CD4⁺ thymocytes was similar in the two types of chimeras. Since steady state numbers of mature thymocytes do not necessarily reflect the rate of generation of those cells, we also analyzed their de novo generation using the postirradiation reconstitution assay (17, 23). While the rate of generation of mature CD8⁺ thymocytes was increased approximately twofold, the rate of generation of CD4⁺ cells in (TCR-α⁺ + B6.PL) → B6 chimeras was similar to that of control (B6 + B6.PL) → B6 mice (Fig. 4, Table I). These data confirm those of the steady state and indicate that the increase in the number of CD8⁺ thymocytes results from an increased generation, rather than thymic retention, of these cells.

**Discussion**

During their development in the thymus, T lymphocyte precursors undergo two selection processes that differ in the requirement for TCR expression: “β selection,” which depends on expression of a pre-TCR, and “positive/negative” selection for which a complete TCR-αβ is required. Using mixed bone marrow chimeras in which only a minority of precursors is selectable, we show here evidence that the differentiation of mature CD8⁺ but not CD4⁺ or immature CD4⁺CD8⁺ thymocytes is limited by homeostatic mechanisms.

β Selection is accompanied by proliferation that is responsible for the generation of most thymocytes, and by an up-regulation of expression of CD4 and CD8. The pre-TCR that mediates β selection contains a monomorphic pT α-chain and a clonotypic TCR.
B6.PL-derived cells were as follows: CD4 gates used for the calculation of percentages of mature thymocytes among control chimera-derived thymocytes. In 2FITC/Thy-1.2-FITC, CD4-PE, and CD8-Red613) 9 to 13 days later. Indi-
chimeras. Six weeks postengraftment the indicated chimeras were suble-
are shown (Thy-1.2-FITC, CD4-PE, and CD8-Red613). The numbers in-
SD, n = 3). The expression of the MHC class I molecule H-2K was used to better identify positively selected thymocytes (9).

β-chain (1), although other “pre-TCR” compositions have been proposed, all of them containing products of genes requiring re-
arrangement for their expression (26). Rearrangement-defective mice contain few or no CD4+CD8+ thymocytes, presumably be-
cause of the failure to produce a pre-TCR (3, 27). However, SCID and RAG+ thymocytes have been shown to express CD4 and CD8 after irradiation (28–30) and SCID thymocytes can complete this differentiation step in the presence of mature T cells (24, 25). De-
spite the presence of practically normal numbers of B6.PL-derived mature thymocytes in (RAG+ + B6.PL) → B6 chimeras, we did not observe significant numbers of RAG+ thymocytes expressing CD4 and CD8. This result indicates that the expression of CD4 and CD8 induced by mature T cells on SCID-derived thymocytes is specific for this mutation (which allows a low level of productive

FIGURE 3. CD4 vs CD8-phenotype of (TCRα+ + B6.PL) → B6 and control chimera-derived thymocytes. In A and B, typical CD4 vs CD8 contour plots of electronically gated B6.PL (Thy-1.2−)–derived thymocytes are shown (Thy-1.2-FITC, CD4-PE, and CD8-Red613). The numbers indi-
cicate the percentages of CD4+CD8+H-2Klow, CD4+CD8+H-2Kbighigh, and CD4+CD8+H-2Kbighigh cells among electronically gated B6.PL-de-


B6.PL) → B6 mice. The degree of expansion occurring during the transition of the CD4+CD8+CD25+ to the CD4+CD8+ stage was identical in mice with high and low numbers of selectable precursors. Therefore, the number of cell divisions occurring at this stage seems to be preprogrammed and is not determined by thymocyte-extrinsic factors. This surprising result raises the possibility that thymus size is controlled at a very early stage of development, i.e., by the number of CD4+CD8+CD25+ thymocytes capable of expressing a pre-TCR. This number, in turn, may be determined by intrathymic proliferation at this or at earlier stages of differentiation, and/or by immigration of precursors into the thymus. Because of the small number of these precursor cells and the lack of precise phenotypic markers for their identification, the mixed bone mar-
row chimeric system used here cannot reliably be used to investigate this issue.

The development of mature thymocytes has been suggested to be under the control of TCR-independent rate-limiting mecha-
nisms. In one report, limiting the proportion of MHC-expressing thymic stromal cells in an in vitro system was shown to result in a proportional reduction in the steady state levels of mature thymocytes (19). The results were suggested to indicate that each thymocyte can interact with a single “selecting niche,” a concept for which additional supporting data have been reported (32). Al-
though very intriguing, these data do not address the question of whether in a normal thymus the number of selecting niches is limiting for the development of mature CD4+ and/or CD8+ thymocytes.

The notion that the number of thymic niches may be a limiting factor in the development of CD8+ thymocytes was originally pro-
posed in a study addressing the kinetics of TCR transgenic (H-Y/H-2D+) thymocyte development (14). In mixed hematopoietic chimeras containing varying ratios of TCR transgenic to wild-type bone marrow cells, the fraction of TCR transgenic cells that matured inversely correlated with their representation in the immature population. It was proposed that a limited number of selecting niches is available for the positive selection of CD8+ thymocytes. It needs to be kept in mind that these results were obtained using TCR transgenic mice with an immature TCR repertoire in which a very high fraction of cells is positively selectable, in contrast to the normal immature TCR repertoire in which only approximately 5% are estimated to be MHC reactive (16). While this high frequency of selectable cells may saturate any putative rate-limiting selection mechanism in TCR transgenic mice, such limitations may play no detectable role in mice with a normal repertoire.

Our data indicate that in mice with a normal immature TCR repertoire the development of CD8+, but surprisingly not CD4+, thymocytes is limited because of the abundance of selectable CD4+CD8+ precursors. In bone marrow chimeric mice in which only approximately 5 to 10% of the CD4+CD8+ thymic precursors can express the TCR-αβ heterodimer and therefore may be positively selected, the proportion of mature CD8+ but not CD4+ cells is 1.5-fold increased as compared with control chimeras. This steady state increase is confirmed by a 2-fold increased rate of de novo generation of mature CD8+ (but not CD4+) cells in these chimeras. Thus, the number of selectable CD8 lineage thymocytes

rearrangements) (31) and does not occur in strictly rearrangement-
deficient RAG1+ thymocytes (3). Therefore, a pre-TCR containing the product of a rearranging gene is absolutely required for pro-
gression to the CD4−CD8+ stage during normal thymocyte
development.

In (RAG1+ + B6.PL) → B6 chimeras, in which the absolute number of precursors capable of differentiation to the CD4−CD8+ stage is strongly reduced, the total number of thymocytes was sig-
ificantly lower than in (B6 + B6.PL) → B6 mice. The degree of expansion occurring during the transition of the CD4−CD8−CD25+ to the CD4−CD8+ stage was identical in mice with high and low numbers of selectable precursors. Therefore, the number of cell divisions occurring at this stage seems to be preprogrammed and is not determined by thymocyte-extrinsic factors. This surprising result raises the possibility that thymus size is controlled at a very early stage of development, i.e., by the number of CD4−CD8−CD25+ thymocytes capable of expressing a pre-TCR. This number, in turn, may be determined by intrathymic proliferation at this or at earlier stages of differentiation, and/or by immigration of precursors into the thymus. Because of the small number of these precursor cells and the lack of precise phenotypic markers for their identification, the mixed bone marrow chimeric system used here cannot reliably be used to investigate this issue.

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apparently saturates the selection mechanism in normal mice while the development of CD4 lineage cells seems to be limited only by the expression of a suitable TCR. Our results may at least partially explain the excess of mature CD4$^+$ over CD8$^+$ thymocytes, although other factors (e.g., immature TCR repertoire) probably also contribute. Moreover, homeostatic limitations in the generation of mature CD8$^+$ (but not CD4$^+$) thymocytes may contribute to the genetically determined differences in CD4/CD8 ratios observed in certain mouse strains and in man (33–35). In any event our results are consistent with data suggesting that immature thymocytes may differentiate into the CD4 lineage by default while additional signals seem to be required for development of CD8 lineage thymocytes (36, 37).

Since homeostatic limitation of mature CD8$^+$ thymocyte development is TCR specificity independent, the responsible mechanism seems to regulate either commitment to the CD8 lineage (41) or survival of already committed thymocytes. It will therefore be of considerable interest to identify the homeostatic mechanisms limiting the development of mature CD8$^+$ thymocytes. In this regard, a putative role of the product of the Notch1 gene in the development of mature CD8$^+$ cells has been invoked (38). A transgenic constitutively activated Notch1 protein was shown to favor differentiation of mature CD8$^+$ thymocytes at the expense of mature CD4$^+$ cells. One explanation for our results could therefore be limited availability of the Notch1 ligand.

The thymic output of mature T lymphocytes is determined by 1) the immigration of bone marrow-derived stem cells into the thymus, 2) the intrathymic generation of T cell precursors, and 3) the proportion of those precursors that will complete maturation. During ontogeny and in case of T cell depletion caused by pathologic conditions (e.g., AIDS), the output of the thymus and thus the concentration of peripheral T lymphocytes could potentially be modulated. As shown in this report, given the right conditions, the rate of generation of mature CD8$^+$ T lymphocytes may be significantly increased. It will be of interest to identify the factors limiting the development of mature CD8$^+$ thymocytes, and to assess whether their natural variations or artificial manipulation will lead to increased thymic output.

Acknowledgments

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References

16. Ignatowicz, L., J. Kappler, and P. Marrack. 1996. The repertoire of T cells shaped by guest on April 17, 2017 http://www.jimmunol.org/ Downloaded from