IL-7 and Not Stem Cell Factor Reverses Both the Increase in Apoptosis and the Decline in Thymopoiesis Seen in Aged Mice

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IL-7 and Not Stem Cell Factor Reverses Both the Increase in Apoptosis and the Decline in Thymopoiesis Seen in Aged Mice

Deborah Andrew and Richard Aspinall

Thymic atrophy is an age-associated decline in commitment to the T cell lineage considered to be associated with defective TCR β-chain rearrangement. Both IL-7 and stem cell factor (SCF) have dominant roles at this stage of triple negative (TN) thymocyte development. Because there is no age-associated decrease in the number of CD44^+CD25^+CD3^-CD4^-CD8^- cells, this study investigated whether alterations in apoptosis within the TN pathway accounted for diminishing thymocyte numbers with age. Here we show significant age-associated increases in apoptotic TN thymocytes, specifically within CD44^+CD25^- and CD44^+CD25^+ subpopulations, known to be the location of TCR β-chain rearrangement. IL-7 added to TN cultures established from old mice significantly both reduces apoptosis and increases the percentage of live cells within CD44^+CD25^- and CD44^+CD25^+ subpopulations after 24 h, with prosurvival effects remaining after 5 days. SCF failed to demonstrate prosurvival effects in old or young cultures, and IL-7 and SCF together did not improve upon IL-7 alone. IL-7R expression did not decline with age, ruling out the possibility that the age-associated increase in apoptosis was attributed to reduced IL-7R expression. Compared with PBS, treatment of old mice with IL-7 produced significant increases in live TN cells. By comparison, treatment with SCF failed to increase live TN numbers, and IL-7 and SCF together failed to significantly improve thymopoiesis above that shown by IL-7 alone. Thus, treatment with IL-7 alone can reverse the age-associated defect in TN thymocyte development revealed by in vitro studies to be located at the stages of TCR β-chain rearrangement. The Journal of Immunology, 2001, 166: 1524–1530.
in IL-7/−/−, IL-7Rα−/−, IL-7Rγc−/−, and c-kit−/− mice (24–27), and c-kit−/− common γ-chain (γc)−/− mice demonstrate complete abrogation of T cell development (27). The reduced development of the early stages of T cell development observed in IL-7/−/−, IL-7Rα−/−, IL-7Rγc−/−, and c-kit−/− mice is similar to that seen with aging. Clearly, both IL-7 and SCF have an important role in the early stages of the developmental pathway, and both may be associated alterations in TN survival could account for the decline in vivo and their impact on thymopoiesis in aged mice.

Therefore, the aim of this study was to investigate whether age-associated alterations in TN survival could account for the decline in thymocyte numbers with age and identify the effect of IL-7 and SCF on the survival of TN cells from aged mice. Finally, this paper investigated the effect of IL-7 and SCF therapy on TN survival in vivo and their impact on thymopoiesis in aged mice.

Materials and Methods

Mice

Normal C57BL/10 mice were obtained from Harlan Olac (Oxfordshire, U.K.) and were maintained in the animal house at the Imperial College School of Medicine in accordance with local rules and regulations.

Purification and culture of CD3−/CD4−/CD8− (TN) thymocytes from young and old mice

Young (2–3 mo) or old (22–26 mo) mice were sacrificed by CO2 asphyxiation, their thymi were removed, and thymocyte cell suspensions were prepared by pressing the tissue through a 100-μm cell strainer (Becton Dickinson, Oxford, U.K.) into RPMI 1640 medium (Life Technologies, Paisley, U.K.) supplemented with 10% FCS (Sigma, Dorset, U.K.). Erythrocytes were lysed using Ortholys (Ortho, Amersham, U.K.), and the total thymocyte number was counted using a hemocytometer.

Thymocytes were incubated for 15 min at 6–12°C with primary Abs; anti-CD3-biotin (clone KT6; Serotec, Oxford, U.K.), anti-CD4 (clone YTS 191.1; Serotec), anti-CD8 (clone YTS 196.2; a kind gift of Dr. B. Roser, Anglia Polytechnic University, Cambridge, U.K.), anti-CD19 (clone 6D5; Serotec), and F4/80 (clone C1A3-1; Serotec) at a concentration of 1 μg 10^6 cells in ice-cold MACS buffer (5 mM EDTA, 1% BSA in PBS). Cells were washed in MACS buffer and then indirectly labeled with goat-anti-IgG MACS microbeads and streptavidin MACS microbeads (Miltenyi Biotec, Bisley, U.K.) for 15 min at 4°C and then negatively selected on a MACS system. The CD3−/CD4−/CD8− (TN) thymocytes were counted and resuspended in RPMI 1640 medium (Life Technologies) supplemented with 10% FCS (Sigma), l-glutamine (200 mM), penicillin (100 U/ml), streptomycin (100 μg/ml) (Sigma), and 5 × 10^−5 M 2-ME (Life Technologies). TN thymocytes (1–2 × 10^6 cells/200 μl) were cultured at 37°C in a 96-well U-bottom plate (Greiner Labortechnik, Glos, U.K.) in the presence of either PBS, IL-7, SCF, or both IL-7 and SCF (PeproTech EC, London, U.K.). Titration experiments were performed showing the percentage of live cells with IL-7 or SCF within a 0–500 ng/ml concentration range, and a concentration of 50 ng/ml, a concentration previously used by Kim (28), was shown to be mid-plateau for IL-7. SCF did not improve the percentage of live cells from control experiments at any concentration within this range; therefore, 50 ng/ml was chosen for convenience.

Analysis of apoptosis and IL-7R expression within TN cultures from young and old mice

For the analysis of apoptosis within the TN thymocyte subpopulations, a four-color FACs analysis was performed at 0, 24 h, and 5 days of culture. Cells were harvested, washed, resuspended in PBS, and stained with anti-CD4−/CD8− (clone IM7; PharMingen, Oxford, U.K.), anti-CD25−/PE (clone 7D4), and control Abs conjugated to APC and R-PE (PharMingen, Oxford, U.K.). For the analysis of the TN thymocyte subpopulations, a four-color FACs analysis was performed at 0, 24 h, and 5 days of culture. Cells were harvested, washed, resuspended in PBS, and stained with anti-CD4−/CD8− (clone IM7; PharMingen, Oxford, U.K.), anti-CD25−/PE (clone 7D4), and control Abs conjugated to CyChrome, FITC, and biotin (PharMingen) for 20 mon on ice. Cells were washed in PBS, resuspended in 1% paraformaldehyde, and analyzed on a Becton Dickinson FACSCalibur within 5 days of fixation on a program acquiring 10,000 cells. All results were analyzed with WinMDI v.2.7 (Scripps Research Institute, La Jolla, CA).

Treatment of old mice with IL-7 and SCF

Groups of male mice aged 22–23 mo were injected s.c. once a day with one of the following treatment regimens: 1) 1 μg of carrier-free recombinant murine IL-7 (R&D Systems Europe, Oxford, U.K.) per day (an approximate concentration of 25 μg/kg/day) in PBS, representing a per diem dose similar to that used previously (29); 2) 4 μg of carrier-free recombinant murine SCF (PeproTech EC) per day (an approximate concentration of 100 μg/kg/day) in PBS, a dose used previously (30); 3) 1 μg IL-7 and 4 μg SCF per day in PBS; and 4) PBS alone. This regimen was followed for 4 days, and on day 5 the animals were sacrificed and the thymi were removed and analyzed.

Purification of apoptotic thymocytes from IL-7− and SCF-treated old mice

Mice were sacrificed, thymi were removed, and thymocytes were counted as described above. Apoptotic cells were selected using the annexin V micro bead apoptotic cell isolation kit (Miltenyi Biotec, Bisley, U.K.) according to the manufacturer’s instructions. For the analysis of the TN thymocyte subpopulation, cells were stained with biotin-conjugated anti-CD4 (clone K6b), biotin-conjugated anti-CD3 (clone K72), streptavidin conjugated to R-PE, anti-CD8-PE (clone 53-6.7), anti-CD25-FITC (clone IM7), and anti-CD25-APC (clone7D4). Control Abs were conjugated to PE, FITC, biotin, or CyChrome. Cells were fixed poststaining with 1% paraformaldehyde in PBS and analyzed on a Becton Dickinson FACSCalibur within 5 days of fixation on a program acquiring 50,000 cells. The results were analyzed using WinMDI v.2.7 (Scripps Research Institute).

The number of live cells in each TN subpopulation was calculated by subtracting the number of apoptotic cells from the total number of each subpopulation.

Statistical analysis

Comparison of samples was conducted using a two-tailed t test for samples with unequal variance using Microsoft Excel software (Redmond, WA). Differences were considered significant for p < 0.05.

Results

Apoptosis within the TN pathway significantly increases with age and is located at the CD44−CD25+ and CD44−CD25− stages of development

To establish whether an increase in apoptosis within the TN pathway occurs with age, TN thymocytes were purified from young and old thymi and identified as follows. Apoptotic cells were detected by binding annexin V to their plasma membrane, live cells by exclusion of annexin V and 7-AAD and dead cells by binding annexin V and 7-AAD. As shown in Fig. 1, there was a significant increase in the percentage of apoptotic TN thymocytes with age. To identify whether this increase could be attributed to a particular stage in TN development, the apoptotic profile within each of the four TN subpopulations was analyzed. Fig. 2 reveals that only the apoptotic profile of the CD44−CD25+ and CD44−CD25− subpopulations significantly differed between young and old animals. The CD44−CD25+ and CD44−CD25− populations demonstrated...
a significant age-associated decrease in live cells and an increase in apoptotic cells, thus locating the age-associated increase in apoptosis to the stages of the TN pathway associated with initiation of TCR β-chain rearrangement.

**Culture with IL-7 significantly reduces apoptosis within TN thymocytes from aged mice**

To examine whether this age-associated increase in apoptosis could be reversed with cytokines known to have a central role at this stage of thymocyte development, TN thymocytes from young and old donors were cultured with IL-7, SCF, or a combination of IL-7 and SCF for 1 and 5 days. Fig. 3 reveals that TN cells from both young and old donors were protected from apoptosis in the presence of IL-7 after 24 h, with protection clearly still evident after 5 days in culture. Cultures with IL-7 established from young donors showed significant increases in live TN cells at 1 (p < 0.05) and 5 days (p < 0.01). This was paralleled by significant decreases in total annexin V+ (cells that have entered apoptosis or died via apoptosis) TN cells at both time points. Cultures established from young donors with IL-7 in combination with SCF also showed significant increases in live TN cells at both time points. This again was paralleled by significant decreases in total annexin V+ TN cells at both time points. Cultures established from old donors followed this same pattern at both time points. However, analysis of both young and old reveals that IL-7 and SCF together did not significantly improve upon IL-7 alone, and culture with SCF alone had no detectable antiapoptotic effect. Tables I and II show the percentage of live TN and annexin V+ TN cells for all individual experiments and clearly demonstrate that all IL-7 cultures show this increase in live TN and decrease in annexin V+ cells compared with control cultures.

**FIGURE 2.** Comparison of the percentages of apoptotic and live cells within each of the four TN subpopulations purified from young (n = 4) and old mice (n = 3). Means and SDs are shown. *, p < 0.05; †, p < 0.01.

**FIGURE 3.** Culture of TN thymocytes purified from young and old donors with PBS, IL-7, SCF, and a combination of IL-7 and SCF for 0, 1, and 5 days. The percentages of TN cells that have entered or died via apoptosis are represented by annexin V-positive cells and live cells by exclusion of annexin V and 7-AAD. Means and SDs are shown for n = 3–4 animals.
Culture with IL-7 reverses the age-associated increase in apoptosis at the CD44<sup>+CD25<sup>−</sup></sup> and CD44<sup>−CD25<sup>+</sup></sup> stages of development

Because culturing with SCF did not differ from culturing with PBS, and a combination of IL-7 and SCF did not differ from IL-7 alone, we have displayed the pattern of apoptosis within the four TN subpopulations in IL-7 and control cultures after 24 h as examples of each to establish whether the prosurvival effect of IL-7 could be located to a certain stage of TN thymocyte development. Fig. 4 reveals that IL-7 added to cultures of TN cells from both young and old animals reduced the percentage of apoptotic cells and increased the number of live cells in all four subpopulations. Most notably, a significant decrease in apoptosis and a significant increase in live cells was observed after 24 h in culture in the CD44<sup>−CD25<sup>+</sup></sup> and CD44<sup>−CD25<sup>−</sup></sup> TN populations in old animals, earlier revealed as the location of the age-associated increase in apoptosis. Although young donors revealed an increase in live cells at the CD44<sup>−CD25<sup>−</sup></sup> and CD44<sup>−CD25<sup>+</sup></sup> TN stages, unlike the old, these values were not significant. Young donors showed significant increases in live cells at the CD44<sup>−CD25<sup>−</sup></sup> and CD44<sup>−CD25<sup>−</sup></sup> stages only. SCF did not demonstrate detectable antiapoptotic effects on any of the four subpopulations, and IL-7 and SCF together did not differ from culture with IL-7 alone (data not shown).

After 5 days of TN culture, IL-7-mediated protection from apoptosis was still clearly evident (Fig. 5). All four TN subpopulations from young and old showed increases in live thymocytes. In the absence of IL-7, live cells are virtually undetectable. Again, SCF failed to demonstrate an antiapoptotic effect on any of the four subpopulations (no live cells were detectable), and all four subpopulations cultured with IL-7 and SCF together did not differ from those cultured with IL-7 alone (data not shown).

**IL-7R expression does not alter with age**

To establish whether the age-associated increase in apoptosis within the TN compartment was the result of reduced IL-7R expression with age, TN from young (n = 6) and old mice (n = 6) were examined for IL-7R expression. The percentage of CD44<sup>−CD25<sup>+</sup></sup> TN thymocytes expressing the IL-7R was 58 ± 7% in young and 74 ± 6% in old mice, revealing a significant increase in expression with age (p < 0.01). Expression in CD44<sup>−CD25<sup>−</sup></sup> TN thymocytes was 39 ± 20% in young and 61 ± 22% in old mice. Although expression in this population is 1.5 times higher in old animals, this was not significant (p = 0.09). Expression within the CD44<sup>−CD25<sup>−</sup></sup> and CD44<sup>−CD25<sup>+</sup></sup> populations did not significantly change with age (22 ± 19% and 15 ± 10% in young, 20 ± 15% and 10 ± 4% in old mice). This result reveals that a decline in IL-7R expression is not responsible for the age-associated increase in apoptosis within the TN compartment. Fig. 6 shows a representative experiment in this series.

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### Table I. Percentage of live TN thymocytes from young and old donors after culture with PBS, IL-7, SCF, and a combination of IL-7 and SCF for 0, 1, and 5 days<sup>a</sup>

<table>
<thead>
<tr>
<th></th>
<th>% Live TN</th>
<th>24 h</th>
<th>5 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>PBS</td>
<td>IL-7</td>
</tr>
<tr>
<td>Young</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>69</td>
<td>26</td>
<td>47</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>46</td>
<td>60</td>
</tr>
<tr>
<td>3</td>
<td>88</td>
<td>43</td>
<td>64</td>
</tr>
<tr>
<td>4</td>
<td>81</td>
<td>47</td>
<td>66</td>
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<tr>
<td>Mean ± SD</td>
<td>79 ± 8</td>
<td>41 ± 10</td>
<td>59 ± 9&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Old</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>72</td>
<td>36</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>79</td>
<td>28</td>
<td>43</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>31</td>
<td>36</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>31</td>
<td>50</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>74 ± 4</td>
<td>32 ± 3</td>
<td>43 ± 7</td>
</tr>
</tbody>
</table>

<sup>a</sup> TN cells that excluded annexin V and 7AAD staining represented live cells. * p < 0.05.

### Table II. Percentage of annexin V<sup>V</sup> TN thymocytes from young and old donors after culture with PBS, IL-7, SCF, and a combination of IL-7 and SCF for 0, 1, and 5 days<sup>a</sup>

<table>
<thead>
<tr>
<th></th>
<th>% Annexin V&lt;sup&gt;V&lt;/sup&gt;</th>
<th>24 h</th>
<th>5 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>PBS</td>
<td>IL-7</td>
</tr>
<tr>
<td>Young</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>31</td>
<td>73</td>
<td>52</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>53</td>
<td>38</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>56</td>
<td>35</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>51</td>
<td>33</td>
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<tr>
<td>Mean ± SD</td>
<td>21±8</td>
<td>58±10</td>
<td>40±9&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Old</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>28</td>
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<td>ND</td>
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<td>2</td>
<td>19</td>
<td>70</td>
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<td>3</td>
<td>28</td>
<td>67</td>
<td>61</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>68</td>
<td>49</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>25±5</td>
<td>67±3</td>
<td>55±6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Annexin V<sup>V</sup> cells represent the percentage of TN cells that have entered or died via apoptosis. * p < 0.05.
IL-7 therapy significantly increases TN thymocytes in vivo

Groups of old mice were treated with IL-7, SCF, IL-7 plus SCF, or PBS to determine whether the results in vitro were echoed in vivo. Treatment of old mice with IL-7 alone revealed a significant increase in the total TN number (Table III). In comparison, treatment with SCF resulted in no change in total TN number, and treatment with IL-7 and SCF together did increase total TN thymocyte number, although not significantly. There was no significant difference between IL-7 and combination treatment in total TN number.

IL-7 therapy significantly increases numbers of live TN thymocytes in vivo

The total number of live cells was calculated by subtracting the numbers of annexin V+ cells from the total numbers of TN cells and from the numbers of each of the four subsets. Compared with PBS-treated controls, IL-7 therapy significantly increased the number of live cells in the TN population (Table III). Because the total TN number and total live TN number after treatment with IL-7 plus SCF did not significantly differ from IL-7 alone, we have displayed the numbers of live cells within the four TN subpopulations in IL-7-treated mice to establish the prosurvival effect of IL-7 and combination treatment in total TN number.

Discussion

Control over the production of thymocytes is exerted by phases of expansion, selection, and apoptosis during development in the thymus, and any changes in these controlling processes will consequently lead to an alteration in the numbers produced. The reduction in thymocyte number and subsequent decline in thymic output seen with age suggested that a change in these controlling processes had occurred.

Here we show for the first time that there is a significant age-associated increase in apoptosis within the TN population that can be pinpointed to the CD44CD25+ and CD44CD25− stages of thymocyte development. These stages express growth factor receptors with an essential role in thymocyte development, the most important of which are c-kit and the IL-7R complex (IL-7Rα/γc).

FIGURE 4. Comparison of the percentages of apoptotic and live cells within the four TN subpopulations after culture for 24 h with PBS (□) or IL-7 (●). Means and SDs are shown for n = 4 animals. The percentage of apoptotic cells is represented by annexin V-positive cells that exclude 7-AAD and live cells by exclusion of both annexin V and 7-AAD. * p < 0.05; † p < 0.01.

FIGURE 5. Comparison of the percentages of live cells within the four TN subpopulations after culture for 5 days with PBS (□) or IL-7 (●). Means and SDs are shown for n = 4 animals.
c-kit is expressed on CD44<sup>+</sup>CD25<sup>−</sup> and CD44<sup>+</sup>CD25<sup>+</sup> TN populations, and expression is lost by the CD44<sup>+</sup>CD25<sup>+</sup> stage (31), whereas the IL-7R is expressed on all four TN populations (32). Evidence for the obligate requirement of both SCF and IL-7 comes from mice doubly deficient in both receptors. These c-kit<sup>−/−</sup>γ<sub>c</sub><sup>−/−</sup> mice show complete abrogation of T cell development, which is not apparent in the single-deficient mutants (27). The limited thymopoiesis present in IL-7<sup>−/−</sup>, IL-7Rα<sup>−/−</sup>, IL-7Rγ<sub>c</sub><sup>−/−</sup> (24–26), and c-kit<sup>−/−</sup> mice (27) has led to the conclusion that SCF and IL-7 act synergistically at the early stages of T cell development (26, 27). This conclusion provided our approach in analyzing the effect of both IL-7 and SCF on the in vitro survival of TN cells from old mice and identifying the effect of IL-7 and SCF treatment on TN survival in vivo as a potential means of reversing age-associated thymic atrophy. Analysis of the thymi in aged mice reveals a bottleneck in thymocyte production between the multipotent stem cell progenitor stage (CD44<sup>+</sup>CD25<sup>−</sup> TN) and their progeny (CD44<sup>+</sup>CD25<sup>+</sup> TN) that have become committed to the T cell lineage. The number of CD44<sup>+</sup>CD25<sup>+</sup> TN thymocytes does not alter with age; however, all subsequent subsets show markedly reduced numbers (18). This age-associated increase in apoptosis at the CD44<sup>+</sup>CD25<sup>−</sup> and CD44<sup>+</sup>CD25<sup>+</sup> TN stages provides some explanation for the decrease in population numbers after the CD44<sup>+</sup>CD25<sup>+</sup> stage of development.

Our results reveal differences in the comparative ability of IL-7 and SCF to maintain TN thymocytes in vitro. IL-7 alone or in combination with SCF promotes TN survival, reducing apoptosis in vitro. Furthermore, our results showed that IL-7 significantly increased live thymocytes and decreased apoptotic thymocytes at the CD44<sup>+</sup>CD25<sup>−</sup> and CD44<sup>+</sup>CD25<sup>+</sup> TN stages from old donors after 24 h in vitro, thus reversing the age-associated increase in apoptosis. SCF failed to demonstrate any detectable antiapoptotic properties in cultures from young or old donors, and a combination of SCF and IL-7 did not improve upon the effect of IL-7 alone. Finally, the ability of IL-7 and IL-7 plus SCF to maintain live cells in culture was more clearly apparent after 5 days in vitro, revealing the essential nature of IL-7 and not SCF as a survival factor. Although SCF/c-kit and IL-7/IL-7Rαγ interactions have been reported to compensate for each other functionally (26, 27), we did not observe synergy with respect to an antiapoptotic effect on TN cells. In the absence of IL-7, SCF does not replace IL-7 as a pro-survival factor in culture. However, we did not investigate other aspects of synergy, such as thymocyte proliferation.

To reinforce the in vitro observation that IL-7 reduces the age-associated increase in TN apoptosis and to identify the impact on survival in vivo, we treated aged mice with IL-7, SCF, and a combination of IL-7 and SCF. IL-7 treatment proved an effective therapy, clearly shown by the significant increase in live TN cell numbers following treatment. The increase in live CD44<sup>+</sup>CD25<sup>−</sup> TN numbers may be due to enhanced survival, enhanced intrathymic proliferation, or increased production of progenitors by the bone marrow and hence increased thymic entry. The increased number of live CD44<sup>+</sup>CD25<sup>−</sup> and CD44<sup>+</sup>CD25<sup>+</sup> TN cells mirrored the changes observed in vitro. Recent work by Kim et al. revealed that the trophic action of IL-7 stopped at the CD44<sup>+</sup>CD25<sup>+</sup> TN stage of fetal thymocyte development and that cell death at the CD44<sup>+</sup>CD25<sup>+</sup> TN stage was independent of IL-7 (28). Our results support this, revealing that IL-7 did not increase live CD44<sup>+</sup>CD25<sup>−</sup> TN cells in aged mice, suggesting that the trophic action of IL-7 at this stage is minimal. The observation that the percentage contribution of live cells to each of the TN subpopulations did not increase with IL-7-treated mice implies that the significant increase in live numbers in vivo is the result of a complex interaction between increased thymopoiesis and the rate of apoptosis.

Having shown that IL-7 induced thymopoiesis in aging animals, we asked whether SCF treatment could induce the same effect. SCF has been shown to prevent apoptosis in various cell types (33, 34), but not in thymocytes. Here we clearly demonstrate that SCF treatment does not increase numbers of live cells in vivo. This result mimics our observations in vitro. The only population to show an increase after SCF treatment in vivo was the CD44<sup>+</sup>CD25<sup>−</sup> TN population, probably reflecting a change in the kinetics of TN development. These results suggest that IL-7 and not SCF is the important contributory factor to the changes seen in the aging thymus, whose deficit may be central to the decline in thymocyte production. TN thymocytes showed no decline with age, either in IL-7Rα expression or in ability to respond to IL-7 in vitro, strongly suggesting that survival of TN cells in the aging thymus is more likely linked to a reduced availability of IL-7 rather than an inability of the cells to respond. In support of this, recent work in our laboratory has demonstrated that expression of intrathymic IL-7 declines in old mice.4

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4 R. Aspinall and D. Andrew. An intra-thymic deficiency in interleukin-7 is linked to age-associated thymic atrophy. Submitted for publication.

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Table III. Mean and SEM of thymocyte numbers in PBS-, IL-7-, SCF-, and IL-7/SCF-treated aged mice

<table>
<thead>
<tr>
<th></th>
<th>PBS-Treated (n = 7)</th>
<th>IL-7-Treated (n = 8)</th>
<th>SCF-Treated (n = 4)</th>
<th>IL-7-+ SCF-Treated (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of TN</td>
<td>1.4 ± 0.3 × 10⁶</td>
<td>3.1 ± 0.5 × 10⁶</td>
<td>1.4 ± 0.2 × 10⁶</td>
<td>2.9 ± 0.9 × 10⁶</td>
</tr>
<tr>
<td>Total number of live TN</td>
<td>1.1 ± 0.3 × 10⁶</td>
<td>2 ± 0.3 × 10⁶</td>
<td>1.2 ± 0.2 × 10⁶</td>
<td>2.2 ± 0.7 × 10⁶</td>
</tr>
</tbody>
</table>

* p < 0.05.
IL-7 plus SCF treatment increases the total number of live TN thymocytes, although not significantly, but failed to improve upon treatment with IL-7 alone. Despite the large increases in total and live TN numbers, the lack of significance in the combination-treated group can be explained by the large SE in this group. There was no significant difference between IL-7 and combination treatment, with the exception of the CD44+CD25+ subpopulation. Here, live cells were significantly lower in the combination-treated mice and may reflect either a change in the kinetics, transit through the group, or the induction of an additional effector molecule affecting this stage of the pathway alone.

The hypothesis explaining that the bottleneck in TN development is due to problems with TCR β rearrangement as a result of reduced intrathymic levels of available IL-7 (18) was questioned recently by a paper showing age-associated thymic atrophy in three strains of mice carrying differing TCR αβ transgenes (35). Unlike the original observations (18), this later study failed to control for the effect of the endogenous transgene by not observing TCR αβ transgenes on a RAG knockout background. The H-Y-transgenic mouse strain mentioned by Lacorazza et al. (35) showing age-associated thymic atrophy does not show atrophy when present on a RAG-2 knockout background (B. Rocha, unpublished observation). Similarly, when mice express the AND transgene (anti-pigeon cytochrome c, class II restricted) on a RAG-2−/− background, age-associated thymic atrophy does not occur (B. Lu, unpublished observation).

In conclusion, the increase in TN apoptosis, the ability of IL-7 to reverse this apoptosis, no decline in IL-7Rα-chain expression, and finally, the fact that intrathymic IL-7 expression declines with age4 all lend support to the concept that thymic apoptosis is due to a deficiency of available intrathymic IL-7 (18). The implication of this work is that therapy with IL-7 is capable of renewing thymopoiesis in old animals, suggesting the possibility that manipulations undertaken to increase the level of intrathymic IL-7, thereby enhancing thymopoiesis, may hold therapeutic potential even in very aged hosts.

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References
6. Thymic atrophy does not occur (B. Lu, unpublished observation). Similarly, when mice express the AND transgene (anti-pigeon cytochrome c, class II restricted) on a RAG-2−/− background, age-associated thymic atrophy does not occur (B. Lu, unpublished observation).