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Hyeyoung Min; ... et. al

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Reduction in the Developmental Potential of Intrathymic T Cell Progenitors with Age[1]

Hyeyoung Min, Encarnacion Montecino-Rodriguez, and Kenneth Dorshkind[2]

Current models of thymic involution propose that intrinsic developmental defects in intrathymic T cell precursors do not contribute to age-related declines in thymopoiesis. This premise was reassessed in a murine model in light of the recent definition of the early T lineage progenitor (ETP), which appears to be the earliest intrathymic precursor defined to date. The results demonstrate that the frequency of ETP declines with age and their potential to reconstitute the thymus is diminished. These findings are consistent with the fact that ETP from aged mice proliferate less and have a higher rate of apoptosis than their counterparts from young animals. Taken together, these data suggest that age-associated changes in T cell precursors should be considered when attempts to rejuvenate the involuted thymus are made. ***The Journal of Immunology*****, 2004, 173: 245–250.**

Involution of the thymus and a decline in T cell production are among the most profound age-related changes affecting the hemopoietic system during senescence (1, 2). These events have been attributed to alterations in the capacity of thymic stromal cells to support thymopoiesis (3, 4) and changes in the production of various endocrine system-derived hormones (5, 6). However, current models of thymic involution indicate that aging does not affect the developmental potential of the most immature intrathymic progenitors. Instead, it has been reported that their development is blocked due to age-related changes in the hemopoietic microenvironment and that the frequency and absolute number of the most immature intrathymic progenitors are increased in the aged thymus (7–10). This model is difficult to reconcile with numerous reports indicating that bone marrow-derived T cell precursors from old mice exhibit declines in number and T lymphopoietic potential (11–14).

T cell progenitors within the thymus are included within the $CD3^-CD4^-CD8^-$ triple-negative (TN)[3] population that comprises ~3–5% of total thymocytes (15). TN cells can be further subdivided, based on the differential expression of cell surface CD25 and CD44, into TN1 ($CD44^+CD25^-$), TN2 ($CD44^+CD25^+$), TN3 ($CD44^-CD25^+$), and TN4 ($CD44^-CD25^-$) subpopulations. The most immature intrathymic progenitors are part of the TN1 compartment (15), and the reported increase in the frequency of intrathymic progenitors with age is based on analysis of the TN1 fraction (7–9).

However, the TN1 compartment is developmentally heterogeneous and includes cells of the myeloid, B, and NK cell lineages (16). Thus, analysis based on the TN1 $CD44^+CD25^-$ phenotype alone may not be sufficient to define the fate of intrathymic T cell progenitors with age. Instead, non-T lineage cells must be rigorously excluded from analysis to yield a fraction of lineage-negative (Lin^-) TN1 cells (Lin^- TN1). Even this level of resolution may not be adequate, because the Lin^- TN1 fraction may still be developmentally heterogeneous. The recent description of a subpopulation of Lin^- TN1 cells with highly enriched T cell progenitor activity, termed the early T lineage progenitor (ETP; Ref. 17), is therefore of importance, because it allows this heterogeneity to be minimized.

The present study describes the properties of ETP from young and old mice and supports a revised model of thymic involution in which age-related, intrinsic defects accumulate in the intrathymic T cell progenitor pool and are a significant contributing factor to thymic involution.

Materials and Methods

Mice

Four- to 6-wk-old male and female C57BL/6J (CD90.2, Thy-1.2) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Timed pregnant Swiss/Webster (CD90.1, Thy-1.1) mice were purchased from Taconic Farms (Germantown, NY). Old C57BL/6J mice, obtained from the National Institute of Aging colony (Bethesda, MD), were at least 17 mo old. Mice were housed in the Division of Laboratory Animal Medicine vivarium at the University of California, Los Angeles.

Fetal thymic organ cultures

Fetal thymic organ cultures were established as described previously (18). Cells were cultured in Iscove's medium supplemented with 15% FCS, 100 μg of streptomycin, 100 U/ml penicillin, 10 μg/ml gentamicin, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, and 5×10^{-5} M 2-ME. Following a 48-h incubation in hanging drop cultures using a Terasaki plate (Nunc, Pittsburgh, PA), cells were cultured an additional 12 days on membrane rafts. Donor cell origin was always confirmed on the basis of CD90 (Thy-1)-allotypic differences. Preliminary kinetic analyses demonstrated that optimal ETP expansion and the development of CD4- and/or CD8-expressing thymocytes occurred at this 2-wk time point (data not shown).

Immunofluoresence and flow cytometry

Thymocytes from adult thymi or fetal thymic organ cultures were immunolabeled with FITC-, PE-, tricolor-, allophycocyanin-, or biotin-conjugated Abs to the following cell surface determinants: CD90.2 (Thy-1.2; clone 53-2.1), CD44 (clone IM7), CD25 (clone 7D4), CD11b (clone M1/70), Gr-1 (clone RB6-8C5), CD45R (clone RA3-6B2), CD3ϵ (clone145-2C11), CD4 (clone RM4-5 or clone GK1.5), CD8α (clone 53-6.7), $\gamma\delta$TCR (clone GL3), $\alpha\beta$TCR (clone H57-597), CD49b/Pan-NK (clone DX5), NK1.1 (clone PK136), CD11c (clone HL3), CD19 (clone 1D3), IgM (clone

Department of Pathology and Laboratory Medicine and Hematopoietic Malignancies Program Jonsson Comprehensive Cancer Center, David Geffen School of Medicine, University of California, Los Angeles, CA 90095

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[2] Address correspondence and reprint requests to Dr. Kenneth Dorshkind, Department of Pathology and Laboratory Medicine, 173216 David Geffen School of Medicine, University of California, 10833 Le Conte Avenue, Los Angeles, CA 90095-1732. E-mail address: kdorshki@mednet.ucla.edu

[3] Abbreviations used in this paper: TN, triple negative; Lin, lineage; ETP, early T lineage progenitor; CLP, common lymphoid progenitor.

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1B4B1), Ter119 (clone Ter119), CD127 (IL-7Rα; clone SB/14), and CD117 (clone 2B8). All Abs were obtained from BD Pharmingen (San Diego, CA) except for anti-IgM (Southern Biotechnology Associates, Birmingham, AL) and Ter119 (eBiosciences, San Diego, CA). Biotin-labeled cells were revealed by incubation with tricolor or allophycocyanin-conjugated avidin (Caltag Laboratories, Burlingame, CA, or BD Pharmingen). The appropriate dilution for each Ab was determined before use.

ETP were identified according to the $Lin^-CD44^+CD25^-CD117^{high}CD127^{low/neg}$ phenotype as described previously (17). The gating parameters used during the separation procedure are described in *Results*. Flow cytometric separations were done using a FACScan (BD Biosciences, San Jose, CA) or FACSVantage (BD Biosciences) located in the Flow Cytometry Core of the Jonsson Comprehensive Cancer Center. The common lymphoid precursor (CLP) 2 was isolated based on its Lin^- TN1 $CD117^-CD45R^+$ phenotype (19).

Detection of apoptotic cells

The frequency of apoptotic cells in freshly harvested thymocyte populations was assessed by labeling with annexin V using the Apoptosis Detection kit as described by the manufacturer (R&D Systems, Minneapolis, MN) in combination with Abs to various cell surface determinants.

Cell proliferation

The proliferative status of cells in the thymus was analyzed by Ki-67 staining (20). Following labeling with Abs to various cell surface determinants, thymocytes were fixed, permeabilized, and stained with an anti-Ki-67 Ab (BD Biosciences) or mouse IgG1 isotype control before analysis on a FACS Calibur. All analyses were performed on freshly harvested thymocytes.

Statistics

Data were analyzed using a two-tailed Student's t test ($\alpha = 0.05$).

Results

Aging reduces the number of immature T cell subpopulations in the thymus

The most immature intrathymic T cell progenitors are included within the $CD3^-CD4^-CD8^-CD44^+CD25^-$ (TN1) population of thymocytes, and it has been reported that the frequency of this population is increased with age (7–10). The data in Fig. 1*A* are consistent with this conclusion and demonstrate that the frequency of TN1 thymocytes is significantly increased in old mice.

However, the TN1 fraction is heterogeneous and it is critical to exclude non-T lineage cells from analysis to estimate more accurately the frequency of intrathymic T cell progenitors. Phenotypic analysis revealed that ~12% of TN1 cells express CD11b (Mac1) and/or Gr-1. The proportion of cells with this phenotype did not change with age (Fig. 1*A*). Approximately one-half of the TN1 cells in young mice express CD45R and >85% of these cells coexpress CD19, indicating their commitment to the B cell lineage (data not shown). The frequency of these $CD45R^+$ cells is slightly increased in old mice (Fig. 1*A*).

When the $CD11b^+/Gr\text{-}1^+$ and $CD45R^+$ cells are excluded from analysis, the frequency of Lin^- TN1 cells was comparable between young and old mice, accounting for ~3–4% of cells within the Lin^- TN compartment (Fig. 1*B*). The total number of TN1 and Lin^- TN1 cells is significantly decreased in old mice due to the dramatic decrease in thymus cellularity (Fig. 1, *C* and *D*, and Table I). However, the Lin^- TN1 cells in old mice constitute a higher proportion of total thymocytes than in the young thymus (Table I).

Similar analysis of TN2, TN3, and TN4 thymocytes in young and old mice indicated that the frequency of cells in these fractions did not significantly change with age (Fig. 1*A*). Because they contained few non-T lineage cells, the frequencies of the Lin^- TN2, Lin^- TN3, and Lin^- TN4 compartments was comparable to that of TN2, TN3, and TN4 fractions, respectively (cf Fig. 1, *A* and *B*). However, the absolute number of TN2, TN3, and TN4 or Lin^- TN2, Lin^- TN3, and Lin^- TN4 cells was significantly decreased in the old thymus due to the severe decline in overall cellularity that accompanies involution (Fig. 1, *C* and *D*, and Table I).

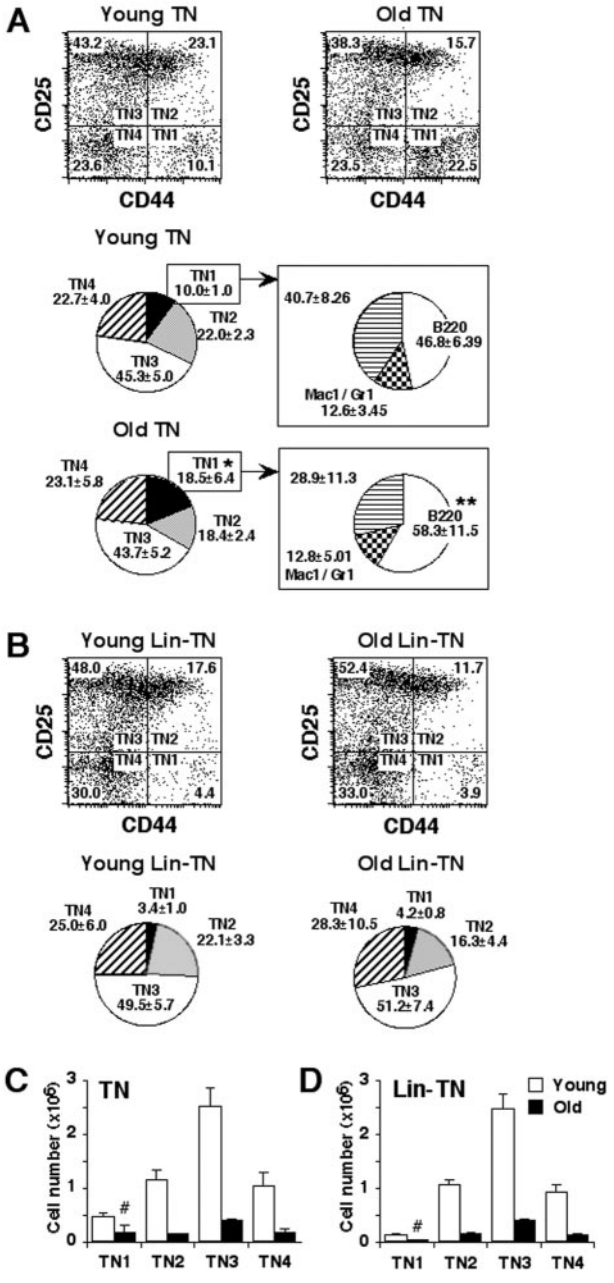

FIGURE 1. Age-related changes in the TN and Lin^- TN thymus compartments. Frequency of TN1, TN2, TN3, and TN4 subpopulations within the TN (*A*) or Lin^- TN (*B*) thymic fractions from young and old mice. The FACS plots show the TN and Lin^- TN compartments from a single young and old mouse while the pie graphs present data pooled from six to seven mice per group. The frequency of $CD45R^+$ and $CD11b/Gr1^+$ cells within the TN1 subset is also indicated. Absolute number of TN1, TN2, TN3, and TN4 subpopulations in the TN (*C*) or Lin^- TN (*D*) fractions from young and old mice. Six young and seven old mice were individually examined and data were pooled to calculate means and SDs. *, $p < 0.01$; **, $p < 0.06$; #, $p < 0.0005$.

ETP have potent thymopoietic potential

Even though Lin^- TN1 cells are a highly purified fraction of thymocytes, they may still be developmentally heterogeneous (16). Therefore, their further fractionation was necessary to assess how aging affects the earliest intrathymic T cell progenitors. In this

Table I. *Frequency and absolute number of intrathymic progenitors in young and old mice*

	ETP			Lin$^-$ TN1			Lin$^-$ TN2			Lin$^-$ TN3			Lin$^-$ TN4		
	%[a]	No.[b]	p[c]	%[a]	No.[b]	p[c]	%[a]	No.[b]	p[c]	%[a]	No.[b]	p[c]	%[a]	No.[b]	p[c]
Young[d]	0.0074 (0.001)	1.36 (0.24)	0.000[e]	0.067 (0.017)	0.49 (0.12)	0.015	0.440 (0.086)	0.09 (0.35)	0.379	0.988 (0.174)	2.21 (0.56)	0.015	0.493 (0.107)	1.10 (0.30)	0.144
Old[d]	0.0014 (0.001)	0.04 (0.03)		0.103 (0.027)	0.15 (0.08)		0.394 (0.093)	0.11 (0.03)		1.243 (0.147)	0.33 (0.07)		0.719 (0.336)	0.18 (0.06)	

[a] Mean percentages of the indicated populations are shown with SDs in parentheses. Each value represents the frequency of indicated population within total thymocytes.
[b] Mean absolute numbers of the indicated populations were calculated by multiplying total thymocyte number by the frequency of cells. SDs are shown in parentheses.
[c] Values of p were calculated for frequencies using a two-tailed Student's t test.
[d] Young mice were 1–1.5 mo old (n = 6–7) and old mice were 19–20 mo old (n = 7).
[e] A value of 0.000 represents $p < 1 \times 10^{-8}$.

regard, a subpopulation of Lin$^-$ TN1 cells highly enriched in T cell developmental potential, termed the ETP, has recently been defined. As shown in Fig. 2*A*, ETP are Lin$^-$ TN1 CD117^{high} CD127$^{low/neg}$ and account for ~0.0074 ± 0.0007% of total thymocytes as reported (Ref. 17; Table I; Fig. 3*A*). ETP have limited myeloid developmental potential (17), and this finding was also confirmed by their culture in semisolid medium with IL-3, IL-6, c-kit ligand (stem cell factor), Epo, and GM-CSF (data not shown). However, the data in Fig. 2*B* confirm that they are extremely efficient at repopulating the thymus as reported elsewhere (17). When 400 ETP were seeded into fetal thymic lobes, they expanded 114-fold. These properties strongly support ETP as being a critical intrathymic progenitor population.

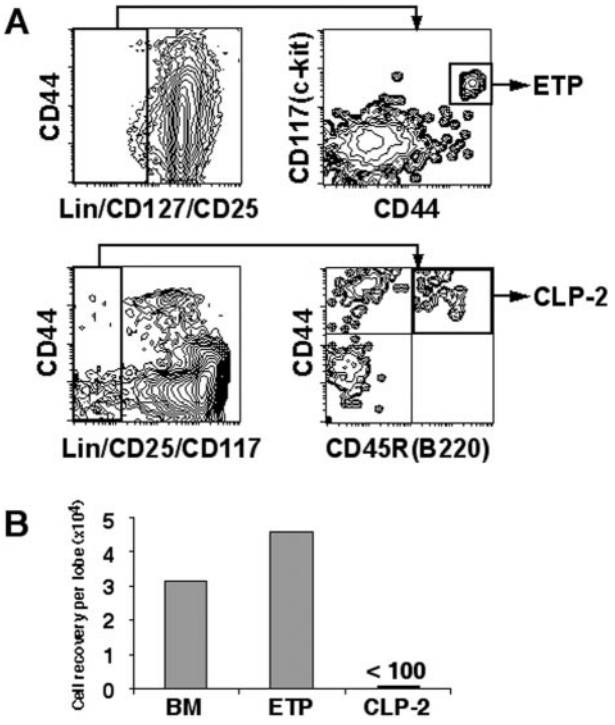

FIGURE 2. ETP have potent thymopoietic potential. *A*, Flow cytometric strategy used to isolate ETP and CLP-2. Abs against CD3ε, CD8α, CD11b, CD45R, Gr-1, γδTCR, αβTCR, NK1.1, and TER119 were used to deplete Lin$^+$ cells for ETP, and Abs specific for CD3ε, CD8α, CD11b, Gr-1, γδTCR, αβTCR, TER119, CD19, IgM, Gr-1, NK1.1, CD49b/Pan-NK, and CD11c were included in the lineage mixture for the isolation of CLP-2. *B*, A single pool of thymic lobes was prepared, subdivided into aliquots, and seeded with 400 ETP, 400 CLP-2, or 5×10^4 unseparated bone marrow cells from young mice. Lobes were processed 2 wk later. The data are representative of three independent experiments with similar results.

Another early intrathymic T cell progenitor population within the Lin$^-$ TN1 fraction has been recently characterized in mice expressing a human CD25 (huCD25) gene under the control of the pre-TCRα promoter. This population, referred to as the CLP-2, is defined as Lin$^-$ TN1 huCD25$^+$CD117$^-$CD45R$^+$ (19), and would be included in the Lin$^-$CD117$^-$CD45R$^+$ TN1 fraction of thymocytes in nontransgenic mice. When isolated according to this phenotype and seeded into thymic lobes, the thymopoietic potential of CLP-2, in agreement with the initial report describing them (19), was minimal. Although a few CD4- and/or CD8-expressing cells were generated (data not shown), CLP-2 expansion was undetectable and only a few hundred cells at most were recovered from lobes (Fig. 2*B*). In view of these data, CLP-2 were not considered in the remaining studies.

ETP frequency and developmental potential are reduced with age

The potent thymopoietic potential of ETP supports the conclusion that they represent a critical progenitor population from which thymocytes are generated. Consequently, the effects of aging on cells in this compartment were determined. As shown in Fig. 3, *A* and *B*, ETP exhibited a significant decline in frequency and absolute cell number with age.

Although their frequency and number are significantly reduced with age, these data do not provide information about the developmental potential of ETP during senescence. Therefore, ETP were isolated from young and old mice and seeded into thymic lobes. Fig. 3*C* further confirms the integrity of our FACS strategy for identification of ETP. When 300 ETP from young mice were seeded into thymic lobes, they efficiently generated progeny that expressed CD4 and/or CD8. In contrast, 300 ETP from old mice generated significantly lower numbers of thymocytes following 2 wk of culture in fetal thymic lobes.

ETP from old mice show reduced proliferation and survival ex vivo

To investigate why ETP are present at a reduced frequency in old mice and do not efficiently generate thymocytes, the effects of aging on their growth and survival were tested. Thymocytes from young and old mice were isolated, ETP were phenotypically identified as described above, and their proliferative and apoptotic status were determined. As shown in Fig. 4*A*, the frequency of ETP that expressed Ki-67, a nuclear Ag found in proliferating cells in late G_1, S, G_2, and M phases of the cell cycle (20), decreased progressively with age and was significantly reduced in cells directly harvested from 20-mo-old mice. In addition, ETP among freshly isolated thymocytes from aged mice exhibited a high level of apoptosis as assessed by annexin V binding (Fig. 4*B*).

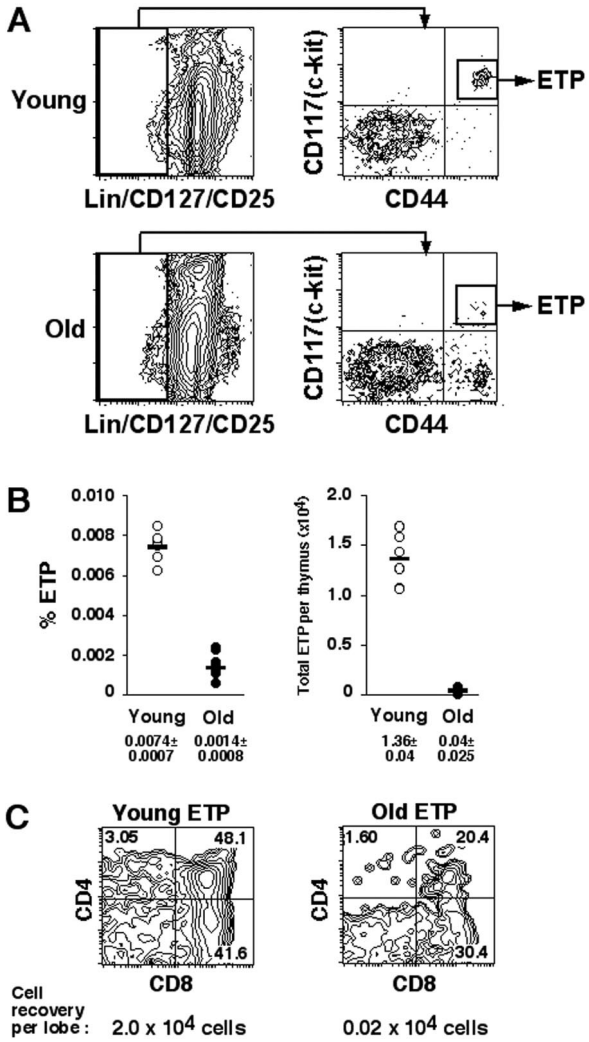

FIGURE 3. ETP number and developmental potential declines with age. *A*, Flow cytometric analysis of ETP from young and old mice. A total of 5×10^5–10^6 viable cells were acquired for analysis after staining for the indicated surface proteins as described. *B*, Frequency and absolute number of ETP from young and old mice. Each circle represents data from an individual mouse and the solid lines indicate means for each group. Absolute number of ETP was calculated by multiplying total thymocyte number by the frequency of ETP. Data are presented as the mean ± SD for $n = 7$ young and $n = 7$ old animals. *C*, ETP were isolated from young and old mice, and 300 cells were seeded into fetal thymic lobes. Two weeks after initiation of cultures, cell recovery and the frequency of donor-derived ($CD90.2^+$) $CD4^-$ and/or CD8-expressing thymocytes was determined. The data are representative of three independent experiments with similar results.

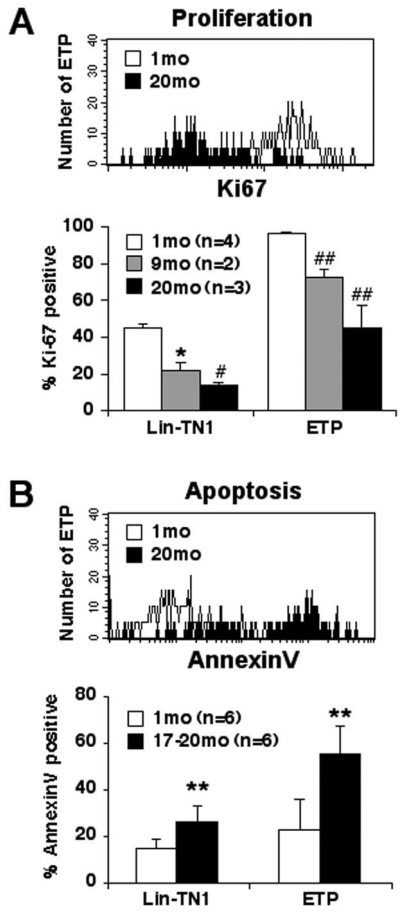

FIGURE 4. Proliferation and survival of ETP from young and old mice. Thymocytes were freshly isolated from young and old mice and stained for ETP in combination with anti-Ki-67 or annexin V labeling to determine their proliferation or survival status, respectively. *A*, ETP were labeled with anti-Ki-67 or isotype control Abs following fixation and permeabilization. The *upper panel* shows representative data for single young and old animals. The *lower panel* indicates data pooled from the indicated number of different aged mice at each time point. *B*, The level of apoptosis in ETP was evaluated by annexin V labeling. The *upper panel* shows representative data for single young and old animals. The *lower panel* indicates data pooled from the indicated number of different aged mice at each time point. Means and SDs are shown for $n = 2$–6 animals as indicated. ∗, $p < 0.02$; ∗∗, $p < 0.002$; #, $p < 0.005$; ##, $p < 0.0005$.

Discussion

The aim of the present study was to determine the effects of aging on the most immature intrathymic T cell progenitors, taking into account the heterogeneity of the TN1 compartment and the recent definition of the ETP (17). Analysis of thymic involution in the context of this recently defined intrathymic progenitor population is advantageous because it excludes from analysis non-T lineage cells known to exist in the TN1 compartment (16). This in turn allows a more accurate assessment of the frequency, absolute number, and developmental potential of T cell progenitors in the thymus. This analysis has resulted in a revised model of thymic involution proposing that the accumulation of age-related developmental defects in T cell progenitors contributes to that process.

Our data are in agreement with previous reports indicating that the frequency of TN1 cells increases with age (7–10). However, because the TN1, and even the Lin^- TN1 fraction, is developmentally heterogeneous (16), further depletion of non-T lineage cells was performed to focus on the ETP. The data demonstrated that ETP frequency and absolute number are significantly reduced by 18 mo of age. It is unknown at what age the decrease in ETP numbers initiates. Thymic involution is a progressive process and accelerates at the initiation of puberty with a marked decline in thymus cellularity thereafter. Thus, it is likely that the decline in the ETP population is also a progressive process that would initiate in mice much younger than the 18- to 20-mo-old cohort examined in this study. In support of this premise are data in Fig. 4 demonstrating that ETP proliferative defects were already observed in mice at 9 mo of age.

The data further indicate that senescence significantly affects the thymopoietic potential of ETP. To define a mechanism that would explain this observation as well as the decline in ETP numbers with age, ETP growth potential and survival were examined. The

data demonstrated that ETP isolated from old animals proliferate to a lesser degree than their counterparts from young mice. In addition, the results indicated that the frequency of apoptotic ETP was higher in the thymus of old animals wherein ~50% of ETP labeled with annexin V. This relatively high frequency of apoptosis among old ETP could indicate that critical intracellular signaling pathways, such as those involving bcl-2, bcl-$X_{L,}$ or p53 might be affected by aging. Alternatively, ETP that do undergo programmed cell death may not be efficiently cleared, either because their location in the thymus is not in proximity to phagocytes or because the function or number of the latter cells is also compromised by aging. Further studies will be required to determine the degree to which intrinsic vs extrinsic effects account for the elevated frequency of apoptotic ETP in the involuted thymus.

In addition to defects in cell growth and possibly survival, additional deficiencies could affect the developmental potential of aged ETP. For example, it is likely that ETP are produced in the bone marrow and their emigration from that tissue to the thymus could be compromised with age. Although the present studies cannot exclude ETP homing defects, preliminary results using thymic reaggregate cultures suggest that these alone are unlikely to explain why aged ETP inefficiently generate thymocytes. When Lin^- bone marrow cells from young and old mice, which would contain ETP and/or their precursors, were cultured with thymic stromal cells in reaggregate cultures, which obviate the requirement for migration into the thymus, old Lin^- cells still exhibited significant T lymphopoietic defects (data not shown).

Taken together, our data are consistent with numerous observations demonstrating that bone marrow precursors from old mice inefficiently generate T lineage progeny (13, 14, 21, 22). In fact, it is possible that age-associated defects in ETP could initiate at a prethymic bone marrow stage of development. For example, Sharp et al. (21) demonstrated that in a competitive repopulation assay in which young and old bone marrow cells were mixed before seeding in the thymus, young cells were more efficient at generating thymocytes. In addition, it is also possible that defects in ETP are further exacerbated by residence in the aged thymic environment (23, 24). These issues aside, the conclusion, based on analysis of the ETP, that aging affects the T cell developmental potential of intrathymic progenitors contrasts with a previous report concluding that Lin^- TN1 cells have normal developmental potential (10). However, careful examination of the FACS plots presented in that study indicates that the TN1 cells used to seed fetal thymic organ cultures were significantly contaminated by a $CD44^{low/neg}$ population and therefore could include cells at the TN4 stage of development. This possibility is further suggested by the fact that thymic reconstitution in these experiments was measured at 6 days after culture initiation. This relatively early time point is used to detect T cell reconstitution by mature pro-T cells, such as those at the TN4 stage of development, but not early progenitors like the ETP (15). Furthermore, the use of highly defined T cell progenitors may mitigate potential positive and negative effects mediated by non-T lineage bystander cells present in the Lin^- TN1 fraction, and allow a more accurate measurement of T cell developmental potential.

In addition to the ETP, an additional early intrathymic progenitor, referred to as the CLP-2 (19), was recently described. We were able to identify a phenotypically distinct population consistent with CLP-2 in B6 mice, but they were extremely inefficient at generating T cells. This result is in accord with findings in the original report describing the CLP-2 (19) and suggests that they are unlikely to be a principal progenitor from which thymocytes are normally produced. This latter hypothesis is consistent with the fact that CLP-2 are derived from CLP-1 (25), which appear to be a cell destined primarily to generate B lymphocytes (26) and express Pax-5, a transcription factor whose activity efficiently blocks T lineage development and further commits progenitors to the B lymphocyte lineage (27). These points do not exclude the possibility that CLP-2 efficiently home to the thymus. However, their primary role could be in some other critical intrathymic process other than generating thymocytes.

Our data indicating that the frequency of ETP declines with age and that their developmental potential is significantly diminished is analogous to what has been reported for the B cell lineage in the bone marrow by Miller and Allman (28). Those investigators demonstrated that the frequency and absolute number of bone marrow CLP-1 are significantly reduced with age and that they exhibit severe proliferative defects. Interestingly, the Miller and Allman study (28) reported that the frequency of pre-pro-B and pro-B cells, which are the immediate downstream progeny of CLP-1, declined significantly in old mice. At first analysis, this finding contrasts with the data herein indicating that the frequency of TN2, TN3, and TN4 cells, the downstream progeny of ETP, do not decline with age despite the reduction in the percentage of ETP. The reason for this difference is likely due to the fact that bone marrow also contains myeloid lineage cells whose number increases as the number of B lineage cells declines with age, resulting in the relative decrease of bone marrow pre-pro-B and pro-B cell frequencies. However, few non-T lineage cells are present in the thymus to fill the void created by a decrease in T lineage cells. As a result, the relative proportion of TN2, TN3, and TN4 cells remains constant within the involuted thymus, although their absolute number declines precipitously. In fact, extrapolating the data of Miller and Allman (28) reveals a similar scenario within the pre-pro-B and pro-B cell compartments; i.e., their relative frequency within the B cell compartment as a whole does not significantly change with age but their absolute number declines significantly. Taken together, the data indicate that aging affects the earliest stages of B and T lymphopoiesis by decreasing the frequency and absolute number of early B (CLP-1) and T (ETP) cell progenitors and, apparently, their transition into downstream pro-B and pro-T compartments. This in turn results in a decline in the number of cells in these downstream compartments, even though their relative proportions remain constant.

In conclusion, these data demonstrate that aging in fact affects the integrity of the earliest intrathymic progenitors. There are at least two implications from the present work. First, the demonstration that age-associated defects accumulate in intrathymic progenitors like the ETP is relevant to understanding how thymic involution progresses. In this regard, it is increasingly appreciated that the organization and maintenance of the thymic microenvironment is dependent upon signals from mature T cells (29–31). Thus, initial reductions in T cell numbers due to age-related defects in ETP could affect thymic stromal cell viability or function, thereby compromising the thymopoietic support potential of the thymic microenvironment. This in turn could fuel a downward spiral in which further declines in T cell production and progressive thymic involution occurs. Second, from a clinical perspective, the data suggest that age-associated changes in T cell precursors must be considered in the context of therapeutic rejuvenation of the involuted thymus.

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