Thymopoiesis in elderly human is associated with systemic inflammatory status

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Abstract Immunosenescence studies of age-related immune system damage focused on clinical lymphopenic situations or androgenic blockade have revealed new insights about adult human immune reconstitution. However, as far as we know, the extent of lymphopoiesis in the thymus of elderly humans remains unclear. To this effect, we have analyzed 65 adult human thymuses (from 36 to 81 years; median age 68.6 years) obtained from patients who underwent cardiac surgery. Our results show a correlation between $CD4^+CD8^+$ double-positive (DP) cells and both the age (inverse) and percentage (direct) of peripheral naive T cells, indicating that the thymus is still able to affect the peripheral lymphocyte pool even in the elderly. We also found significant correlation between the degree of thymopoiesis and the inflammation markers, as shown by the inverse correlations between DP and the percentage of neutrophils and IL-6 levels and the percentage of peripheral lymphocytes. Further-

more, in a multivariate linear regression the percentage of DP and IL-7 levels, but not age, were independently associated with the percentage of neutrophils. In conclusion, the thymus maintains, even in the elderly, an active thymopoiesis that rejuvenates the peripheral naive T-cell pool. Moreover, age-related thymopoietic decay is associated with the peripheral inflammation markers.

Keywords Ageing · T cell homeostasis · Human thymus · Immunosenescence · Neutrophil · Systemic inflammation

Introduction

Age-related changes of the immune system (immunosenescence) are complex processes affecting both the adaptative and the innate immune functions (Delarosa et al. 2006; Aw et al. 2007). Among the clinical consequences of the immunosenescence are an increase in the frequency of infectious diseases (and, possibly, an increase in cancer and autoimmune diseases) (Yoshikawa 2000; Strindhall et al. 2007) and poor response to vaccines (Gruver et al. 2007). The loss of peripheral blood naive T cells is the best-known effect of immunosenescence and the absence of response to vaccines has been ascribed, at least in part, to this decline (Aspinall et al. 2007; Gruver et al. 2007). The peripheral naive T-cell pool is maintained, preferably, by thymic lymphopoiesis as well as by the

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homeostatic mechanisms that account for the peripheral expansion (Cambier 2005; Cicin-Sain et al. 2007). In this way, it is generally accepted that age-related thymic involution is the major mechanism involved in naive lymphocyte loss and, therefore, in immunosenescence.

The interest in human thymic function in the elderly has increased after studies showed that (1) the adult thymus of HIV-infected patients without treatment (McCune et al. 1998) or under highly active antiretroviral therapy (HAART) (Franco et al. 2002; De la Rosa and Leal 2003) maintained or even improved its function, actively contributing to the CD4 lymphocyte repopulation; (2) adult thymopoiesis can be increased with growth hormone administration (Goldberg et al. 2007) or pharmacological androgenic blockade (Sutherland et al. 2005; Goldberg et al. 2007b). Therefore, the capacity of effectively reversing, at least partially, the thymic atrophy opens up new perspectives in order to delay the immunosenescence process. In this way, to contribute to augment the information on the thymopoiesis magnitude in elderly people is especially outstanding. In fact, although some authors point to a remaining functionality of the human adult thymus (Shanker 2004; Pawelec et al. 2006), laboratory data are scarce and indirect approximations are mostly used (Marusic et al. 1998). Moreover, few studies have been performed directly on elderly human thymic samples, and most of them used small cohorts, which usually blended ex-vivo analysis with thymic tissue obtained from autopsies (Jamieson et al. 1999; Marinova 2005).

Jamieson et al. (1999) reported that thymopoietic potential per cell, as measured by the quantification of T-cell receptor excision circles (TRECs), remains constant up to 56 years and those thymocytes recognize a broad range of antigens. The maintenance of the thymopoietic potential per cell was subsequently confirmed in both mice (Sempowski et al. 2002) and humans (Sempowski et al. 2000; Gruver et al. 2007). Nevertheless, none of these works assessed a direct association between thymic function-related markers and peripheral naive subsets. Furthermore, it has been reported (Jamieson et al. 1999; Poulin et al. 1999) that new T lymphocytes continue to emigrate from the thymus even in adulthood, yet, as far as we know, whether this output is large enough to modify the peripheral naive T-cell pool through an effective lymphopoiesis remains unclear.

Regarding the innate immune system, many altered functions and inflammation markers (Gomez et al. 2005; De Martinis et al. 2006) predicting frailty in the elderly have been reported. The failure in defenses against bacteria might indicate that the function of neutrophils is seriously impaired in the elderly (Lord et al. 2001). Moreover, the rise of the pro-inflammatory cytokines [interferon (IFN) γ , tumor necrosis factor (TNF) α , interleukin (IL)-1 or IL-6] and decrease of the anti-inflammatory cytokines (IL-10, IL-4, IL-5) show a systemic inflammation status. Despite both innate and adaptative immune responses being known to be affected in the elderly (De Martinis et al. 2006), the relationships between the two processes have not yet been described.

In this context, the aim of this study was to analyze the extent of the remaining thymus functionality in old people and whether this thymic function correlated with the systemic inflammation observed in the elderly.

Materials and methods

Patients This study included 65 consecutive patients who underwent cardiac surgery (valvular repair or ischemic cardiopathy) at Virgen Del Rocio University Hospital in Seville, Spain, between February 2006 and August 2007. Thymic tissue and peripheral blood samples were collected from each individual. Patients had not received any treatment that could influence their immune status, such as radiation (Mackall et al. 1995), steroids (Cohen et al. 1992) or antitumor therapy (Kumamoto et al. 1995); there were also no clinical data of active infections, including an HIV negative test.

Thirty-five additional healthy, elderly volunteers (median age 81 years, IQR 78–88) were included as a control group. Simultaneous plasma and serum samples were collected and frozen at -20°C until further use. This group was only used when comparing IL7 serum and plasma values.

All individuals were informed and consented to participate in the study, which was approved by the Ethical Committee of the Hospital.

Thymocytes, peripheral blood mononuclear cells (PBMCs) and serum isolation Thymic tissue samples were extracted during cardiac surgery and processed

immediately. Samples and tissues were only obtained for clinical reasons, and never for research purposes. The aged thymus remained clearly distinguishable despite the adipose tissue of the mediastinum, but we could not ensure that all the remaining thymic tissue was removed. Nevertheless, identification and representative sample taking were guaranteed (Fig. 1a). Thymic tissue samples were washed with phosphate-buffered saline (PBS) and then disaggregated with a wire mesh in RPMI medium. Thymocytes were isolated by Ficoll-Hypaque density gradient and washed with PBS. The total counts of isolated cells were considered as the cellularity of the thymic tissue sample.

PBMCs were isolated from whole blood in Vacutainer CPT tubes with sodium heparin as an anticoagulant and cryopreserved in liquid nitrogen (fetal bovine serum, 10% DMSO) until further use. Serum was collected in Vacuette Serum Collector tubes without any anticoagulant and then stored at -20°C until quantification of interleukins.

Absolute cell counts Lymphocyte subpopulations (CD3^+ , CD4^+ and CD8^+ absolute numbers) were determined in fresh whole blood using the Epics XL-MCL (Beckman-Coulter, Calif., USA) flow cytometer, according to the manufacturers' instructions. Absolute numbers and percentages of lymphocytes, monocytes and neutrophils were extracted from the hemogram

performed in fresh whole blood in the Sysmex XE-2100 (Roche Diagnostics, Ind., USA) cell counter.

IL-6 and IL-7 quantification The determination of IL-6 and IL-7 sera and plasma levels was performed in duplicate using a highly sensitive colorimetric enzyme-linked immunosorbent assay (IL-6 and IL-7 Quantikine ELISA kit, R&D Systems, Minneapolis, Minn., USA), according to the manufacturers' instructions. A calibration curve was constructed using mean absorbance obtained from each standard in duplicate. The cytokine concentration of each duplicate sample was then extrapolated from the standard curve and then calculated using the mean value.

Flow cytometry The frequency of $\text{CD4}^+\text{CD8}^+$ double-positive (DP) thymocytes among fresh whole thymocytes was determined by flow cytometry using anti-CD4 FITC and anti-CD8 PE monoclonal antibodies (Becton Dickinson, San Jose, Calif.). Thymocytes were selected by the forward and scatter profiles and DP percentage was calculated from this population. The frequency of naive CD4 T cells ($\text{CD4}^+\text{CD45RA}^+\text{CD27}^+$) and naive CD8 T cells ($\text{CD8}^+\text{CD45RA}^+\text{CD27}^+$) was analyzed in frozen PBMC samples using anti-CD45RA FITC, anti-CD27 PE, anti-CD4 PerCP and anti-CD8 PerCP monoclonal antibodies (Becton Dickinson, San Jose, Calif.).

Fig. 1 **a** Representative procedure for the thymic tissue surgically removal. Note the total absence of adipose tissue in the mediastinum that ensures the clear thymic tissue sample identification. **b** Representative histological data for the 0% DP thymic tissue samples. Photographs show an organized lymphoepithelial structure and the black arrow shows a Hassall's corpuscle, both typical of thymic tissue samples

Conventional histology In order to confirm whether the 0% DP samples were true thymic tissue specimens, representative samples were analyzed by conventional histology. One-centimeter thymic pieces were embedded in paraffin wax, then cut in 3- μ m sections, and routinely stained with hematoxylin and eosin (HE) for histological examination.

Quantification of signal-joint (sj) TRECs DNA was directly isolated from thymic tissue samples using the Nucleospin tissue kit (Machery-Nagel). Delta-deletion TRECs formed by δ Rec- ψ J α rearrangement were amplified and quantified by real-time PCR using fluorescently labeled oligonucleotides as reporter probes in a 20- μ l PCR reaction using the Light-Cycler (Roche Diagnostics, Ind.) as previously described (Douek et al. 1998). TREC abundance was normalized to cell number determined by amplification of β -globin with the GH20 and PC04 primers (Bauer et al. 1991). Serial dilutions of the standards, including plasmid clones containing TRECs and β -globin, were run to generate standard curves for further quantification. Data were expressed as TRECs per 10^6 cells using mean values from triplicate assays for TRECs and duplicate assays for β -globin.

Statistical analysis Continuous variables were expressed as median [interquartile range (IQR)], and categorical ones as number of cases (percentage). The Pearson test was used to analyze correlations between Gaussian variables and the Spearman test was used to analyze correlations between non-Gaussian variables. The Mann Whitney *U*-test was used to analyze differences between continuous variables. To assess the potential variables associated with the percentage of neutrophils, those variables from the univariate analysis were introduced into the multivariate forward stepwise multiple linear regression analysis. Statistical analysis was performed using the Statistical Package for the Social Sciences software (SPSS 14.0, Chicago, Ill., USA).

Results

Analysis of the thymic function Cohort characteristics are summarized in Table 1. Thymic tissue samples were analyzed in the 65 individuals of our cohort.

Table 1 Characteristics of the cohort

Demographic parameters (median [IQR])	
Number of patients	65
Age (years)	68.6 [59.1–72.6]
Sex (% males)	40 (61.5)
Thymic parameters (median [IQR])	
Isolated thymocytes ($\times 10^6$)	4.5 [1.5–14.0]
CD4 ⁺ CD8 ⁺ DP thymocytes (%)	31.8 [11.5–49.1]
CD4 ⁺ CD8 ⁺ DP absolute counts	1,374,300 [131,900–6,618,000]
sjTRECs/ 10^6 cells	52,493 [1,189–139,248]
sjTRECs absolute counts	250,579 [2,079–2,785,020]
Blood parameters (median [IQR])	
CD4 T cells (cells/ μ l)	625.5 [405.5–808.3]
CD8 T cells (cells/ μ l)	298.5 [192.0–436.5]
CD4 ⁺ CD45RA ⁺ CD27 ⁺ naive T cells (%)	16.6 [6.9–26.2]
CD8 ⁺ CD45RA ⁺ CD27 ⁺ naive T cells (%)	7.9 [5.1–10.7]
Lymphocytes (%)	28.1 [20.7–37.2]
Neutrophils (%)	58.3 [48.6–68.5]
Neutrophils ($\times 10^9$ /l)	3.9 [3.0–5.1]
IL-7 (pg/ml)	13.56 [10.05–19.22]
IL-6 (pg/ml)	2.04 [1.04–4.94]

Despite visual identification of the thymic tissue samples being clearly determined (Fig. 1a), in order to discard a lymphocyte infiltration on adipose tissue, histological analyses of representative thymus specimens were performed. Conventional histology performed in thymus samples with 0% DP thymocytes showed Hassall's corpuscles and a clearly organized lymphoepithelial structure (Fig. 1b), indicating atrophied but true thymic tissue samples.

DP thymocytes were analyzed from each sample as a thymic function surrogate marker (Fig. 2a). As expected, thymic function, measured either by the percentage of DP thymocytes or DP absolute numbers, declined with age ($r=-0.323$, $p=0.009$ and $r=-0.319$, $p=0.011$, respectively). Nevertheless, both the low correlation coefficient and the DP range (minimum: 0.0% DP, and maximum: 70.5% DP) showed a high heterogeneity degree among individuals. In order to confirm this heterogeneity, we stratified the cohort according to age groups, which showed a high heterogeneity among them (Fig. 2b). In spite of medians being reduced with age, we found individuals with a very high percentage of DP (>50%) in all

Fig. 2 **a** Representative fluorescent-activated cell sorter (FACS) analysis of fresh isolated thymocytes. The plot shows the percentage of DP (CD4⁺CD8⁺) thymocytes. **b** Percentage of DP thymocytes among the different age groups. *Black lines* define the median of DP thymocytes percentage. **c** sjTREC numbers (directly obtained from tissue samples DNA) among

the different age groups. *Black lines* define the median of sjTREC/10⁶ cells. **d** Relationship with the total numbers of isolated thymocytes from each thymus tissue sample. *Black circles* represent the direct correlation ($r=0.525$, $p<0.001$) with the percentage of DP thymocytes. *White circles* show the direct correlation ($r=0.363$, $p=0.004$) with the sjTREC numbers

groups. In the same way, some individuals without thymopoiesis (DP<5%) could be observed in all the groups of individuals over 50 years old (Fig. 2b).

We also examined the sjTREC numbers in thymic tissue samples. Although the TREC/10⁶ cells was age-independent ($r=-0.033$, $p=0.799$), the absolute TREC numbers declined in an age-related manner ($r=-0.248$, $p=0.056$), close to statistical significance. Moreover, the absolute TREC numbers showed a strong direct correlation with the absolute DP numbers

($r=0.615$, $p<0.001$). Regarding heterogeneity, a wide range of TREC numbers, directly quantified in DNA tissue samples, was also found in the age groups (Fig. 2c).

Finally, we analyzed whether the number of cells that we were able to obtain from each sample was related to the thymic function parameters, i.e., DP and sjTREC. Figure 2d shows a significant direct correlation for both the percentage of DP thymocytes ($r=0.525$, $p<0.001$) and TREC numbers ($r=0.363$, $p=$

0.004) with the number of cells isolated from each thymus specimen.

Relationship between thymic and peripheral cell parameters Naive ($CD45RA^+CD27^+$) T cells were analyzed. Percentages of both $CD4^+$ ($r=0.318$, $p=0.002$) and $CD8^+$ ($r=0.363$, $p=0.007$) naive T cells showed a strong direct correlation with the DP absolute numbers (Fig. 3a). The direct correlation was also found by analyzing TREC levels in the thymus, as well as the DP numbers, as a thymic function surrogate marker. Figure 3b shows a significant association between $CD4^+$ ($r=0.263$, $p=0.046$) and $CD8^+$ ($r=0.306$, $p=0.026$) naive T cells from

peripheral blood and the sjTRECs numbers obtained directly from thymic tissue DNA. TRECs/ 10^6 cells also showed a strong direct correlation with both the $CD4^+$ ($r=0.369$, $p=0.004$) and the $CD8^+$ ($r=0.404$, $p=0.002$) naive T-cell subsets. Results support a direct role of the remaining thymic function in elderly and naive T-cell peripheral pool maintenance.

Systemic inflammation markers A slight lymphopenia and a systemic inflammation status are commonly found in elderly people. IL-7 was analyzed as the cytokine involved in the lymphocyte homeostasis and the thymopoietic control. Neutrophils and IL-6 levels were analyzed as peripheral blood inflammation

Fig. 3 **a** Relationship between DP thymocyte absolute numbers and the percentage of naive T cells in peripheral blood. *Black circles* show the correlation with $CD4^+$ naive T cells ($r=0.318$, $p=0.002$, $n=61$), while *white circles* show the correlation with $CD8^+$ naive T cells ($r=0.363$, $p=0.007$, $n=56$). **b** Relationship between the sjTREC absolute counts (obtained

from DNA thymic tissue) and the percentage of naive T cells in peripheral blood. *Black circles* represent the correlation with the $CD4^+$ naive T cells ($r=0.263$, $p=0.046$, $n=60$), while *white circles* represent the correlation with the $CD8^+$ naive T cells ($r=0.306$, $p=0.026$, $n=55$)

markers and the association with the age and the DP thymocytes was studied. The percentage of neutrophils and the IL-7 levels significantly increased with age, while the percentage of lymphocytes decreased in an age-dependent manner (Table 2). Neutrophil percentages also showed statistically significant correlations with the DP thymocytes quantified either as frequency ($r=-0.362$, $p=0.003$) or absolute numbers ($r=-0.381$, $p=0.014$). In addition, the neutrophil percentages showed significant correlation with IL-7 levels ($r=0.415$, $p=0.002$, Fig. 4a). In a multiple linear regression, the percentage of DP thymocytes and IL-7 levels ($p=0.002$), but not age, were independently associated to the percentage of peripheral blood neutrophils (Table 3).

Due to sample availability, IL-7 quantification was performed in serum samples. Despite all samples being processed similarly, IL-7 values could be overestimated, as platelets are known to release IL-7 during clotting. In order to assess the correlation between sera and plasma levels, we analyzed samples from 35 healthy elderly volunteers. In spite the short number of samples analyzed, results showed a linear correlation, close to statistical significance, between both IL-7 values (Fig. 4b). Thus, despite their higher values, IL-7 levels on sera samples maintain the tendency and the biological sense.

Although IL-6 levels did not correlate with absolute DP counts ($r=-0.185$, $p=0.207$), we found a significant inverse correlation between IL-6 concentrations and the percentage of peripheral lymphocytes ($r=-0.365$, $p=0.009$). Moreover, IL-6 concentrations showed a significant correlation with the percentage of peripheral neutrophils ($r=-0.361$, $p=0.009$). Both correlations supported a rise in the systemic inflammation status in a situation of increased lymphopenia.

Table 2 Relationship between age and DP with the percentage of neutrophils and lymphocytes, IL-7 and IL-6 levels

	Age	DP percentage	DP absolute numbers
Neutrophils (%)	$r=0.358$ $p=0.003$	$r=-0.362$ $p=0.003$	$r=-0.381$ $p=0.014$
Lymphocytes (%)	$r=-0.389$ $p<0.001$	$r=0.348$ $p=0.005$	$r=0.356$ $p=0.005$
IL-7	$r=0.415$ $p=0.002$	$p=0.321$	$p=0.115$
IL-6	$p=0.057$	$p=0.282$	$p=0.207$

In order to analyze individuals regarding the systemic inflammatory status, we divided our cohort into neutropenic ($<2.5 \times 10^9/l$), normal and neutrophilic ($>7.5 \times 10^9/l$) status. A significant difference was found between DP percentages ($p=0.006$, Fig. 4c), DP absolute numbers ($p=0.012$) and IL-7 levels ($p=0.005$) among individuals with normal or neutrophilic counts. A significant difference was also found between neutropenic and neutrophilic individuals regarding the DP frequency ($p=0.002$, Fig. 4c) and the DP absolute numbers ($p=0.022$). No differences were found regarding age. As expected, significant differences were found between individuals with neutropenic and neutrophilic counts when analyzing the IL-6 levels ($p=0.021$, Fig. 4d).

Discussion

The results obtained in this work show a high degree of heterogeneity in the adult human thymus function when both DP thymocytes counts and intrathymic sjTREC levels are measured. In our cohort, the remaining thymic function directly correlates with the percentage of naive T cells in both the CD4⁺ and CD8⁺ subsets. We found that the percentage of neutrophils in adult individuals was independently associated with their percentage of DP and the IL-7 serum levels on analyzing systemic inflammation parameters.

A limitation of this study is the bias towards patients that underwent cardiac surgery. The difficulty in obtaining thymic tissue samples forced us to limit to a cohort with a median age of 68.6 years, which reflects the age of patients who underwent cardiac surgery at our hospital. Human thymus studies usually use an indirect measure, such as the TREC quantification in peripheral blood as a thymic function-related marker (Jamieson et al. 1999; Poulin et al. 1999), but in this study, we have analyzed the percentage of DP thymocytes as the thymic function direct marker. In addition, we have directly quantified the TREC levels in thymic tissue DNA, where no proliferation induced by activation occurs. Using direct thymic function-related markers, we have overcome the limitation of using TRECs in peripheral blood for the thymic function quantification (Hazenberget al. 2000; Harris et al. 2005).

The age-related atrophy process of the thymus and its output reduction during adulthood is well established in the literature. In our study, we wanted to

Fig. 4 **a** Direct correlation between the percentage of neutrophils and the IL-7 levels obtained from serum samples ($r = 0.415$, $p = 0.002$). **b** Relationship between serum and plasma IL-7 levels ($r = 0.266$, $p = 0.122$). This IL-7 quantification has been performed in a control group of healthy elderly. **c** DP thymocytes percentage medians among the three neutrophil

count groups. Statistical differences were found between the normal and neutrophilic count group ($p = 0.006$). **d** IL-6 concentration among the neutrophil count groups. Statistical differences were found between the neutropenic and neutrophilic count group ($p = 0.021$)

Table 3 Univariate and multivariate analyses (RC regression coefficient, CI confidence interval)

Parameters	Percentage of neutrophils			
	Univariate		Multivariate	
	p value	p value	RC	95% CI
Age (years)	0.002	0.805		
Percentage of DP	0.003	0.005	0.376	0.461 to 2.030
IL-7 (pg/ml)	0.002	0.034	-0.276	-0.348 to -0.014

analyze the remaining functionality in the elderly human thymus once individuals showed an involuted organ. Half our cohort had more than 31.8% of DP thymocytes. Despite the percentage of DP declining with age, we found a broad distribution in all age groups. Results showed a high heterogenic degree (confirmed with the sjTREC quantification) in the residual thymic function. TREC/ 10^6 cells did not correlate with age in our cohort, possibly because the TREC quantification had been performed directly in tissue DNA. The presence of large amounts of adipose tissue in the thymus is the main marker of atrophy and thymocytes are therefore the main cells contributing to the extracted DNA from each thymic sample. Our results showed that the percentage of DP diminishes with age, but not the TREC levels for million of thymocytes. According to that, TREC absolute numbers show an age-related decrease proximal to statistical significance (0.056). We found a strong direct correlation in both the $CD4^+$ and the $CD8^+$ T-cell subsets on analyzing the relationship between the absolute numbers of DP and the peripheral naive T-cell percentages from each individual. Recently, a high interindividual heterogeneity regarding the $CD4^+$ T-cell counts in adulthood (Kilpatrick et al. 2008) has been reported. Their results suggested age-related differences regarding the peripheral homeostasis, but our results could also help to explain the naive T-cell heterogeneity. Thus, both peripheral homeostasis and DP differences would be denoting a different interindividual maintenance of the naive T-cell pool.

Another well-known age-related immune change is in the systemic inflammation status of the elderly (Gomez et al. 2005; De Martinis et al. 2006). Once

we assessed the thymic functionality and its impact on peripheral T-cell pools, we decided to analyze the inflammation status of our cohort. The infectious diseases prevalent in the elderly (e.g., pneumonia) point to a failure in numbers and/or function of the neutrophil subset (Lord et al. 2001; Targonski et al. 2007). We found that the numbers of neutrophils in the study correlated with (1) percentage of DP, (2) IL-7 levels, and (3) age; but multiple linear regression showed that DP and IL-7 were the only independently associated. Furthermore, we found that neutrophilic individuals showed fewer DP but higher IL-7 and IL-6 levels when stratifying our cohort into neutropenic ($<2.5 \times 10^9/l$), normal or neutrophilic ($>7.5 \times 10^9/l$) status. Unfortunately, in our study IL-7 levels could be overestimated since the platelets had not been previously isolated in order to avoid their IL-7 contribution to the total levels. Although this limitation, Fig. 4b shows the relationship between sera and plasma IL-7 levels in a healthy control group. Thus, our results are internally valid with respect to comparison among our populations, although these IL-7 levels cannot be extrapolated or compared with other studies. Furthermore, the decrease in the percentage of lymphocytes and the increase in the percentage of neutrophils in peripheral blood correlated with the rise of IL-6 levels, supporting the idea that the lymphopenic status correlates with an augment in the systemic inflammation status in the elderly.

All these results could be explained by inter-relating the innate and adaptative immunosenescence processes instead of the independent processes. Despite the mature neutrophils not having a functional IL-7 receptor (Girard and Beaulieu 1997), it has

Fig. 5 Outline showing the relationship between the innate and adaptative immunosenescence processes

been reported that myelopoietic precursors could have (Grzegorzewski et al. 1994). An increase of neutrophils in lymphopenic mice has also been reported after recombinant rh-IL-7 administration (Jiang et al. 2005; Aiello et al. 2007). The increase of IL-7 levels induced by a lymphopenic status is well known. The age-related loss of thymic function could explain the lymphopenic induction that increases IL-7 levels in peripheral blood. IL-7 could be interacting with the myeloid precursors and, thus, could be inducing the increase in the neutrophil levels. The direct correlation between the percentage of neutrophils and the percentage of DP with the percentage of lymphocytes ($r=-0.974$, $p<0.001$) and IL-7 levels, in our cohort of adult people, suggests the hypothetic model summarized in Fig. 5, although an independent phenomenon can not be excluded.

Taken together, our results suggest that the thymus in the elderly is still functional and contributes to the maintenance of the peripheral pool of naive T lymphocytes, despite the interindividual heterogeneity. Furthermore, we show that the thymic function loss in the elderly is directly associated to the neutrophilic status. Further studies are necessary to improve our understanding of the variability among individuals during immunosenescence, and the mechanisms that cause the neutrophil to raise. Moreover, whether the thymic functionality of elderly individuals may be pharmacologically increased remains to be determined.

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References

- Aiello FB, Keller JR, Klarman KD et al (2007) IL-7 induces myelopoiesis and erythropoiesis. *J Immunol* 178(3):1553–1563
- Aspinall R, Del Giudice G, Effros RB et al (2007) Challenges for vaccination in the elderly. *Immun Ageing* 4(1):9. doi:10.1186/1742-4933-4-9
- Aw D, Silva AB, Palmer DB et al (2007) Immunosenescence: emerging challenges for an ageing population. *Immunology* 120(4):435–446. doi:10.1111/j.1365-2567.2007.02555.x
- Bauer HM, Ting Y, Greer C et al (1991) Genital human papillomavirus infection in female university student as determined by PCR-based method. *JAMA* 265:472–477. doi:10.1001/jama.265.4.472
- Cambier J (2005) Immunosenescence: a problem of lymphopoiesis, homeostasis, microenvironment, and signaling. *Immunol Rev* 205:5–6. doi:10.1111/j.0105-2896.2005.00276.x
- Cicin-Sain L, Messaoudi I, Park B et al (2007) Dramatic increase in naive T cell turnover is linked to loss of naive T cells from old primates. *Proc Natl Acad Sci USA* 104(50):19960–19965. doi:10.1073/pnas.0705905104
- Cohen JJ, Duke RC, Fadok VA et al (1992) Apoptosis and programmed cell death in immunity. *Annu Rev Immunol* 10:267–293. doi:10.1146/annurev.iy.10.040192.001411
- De la Rosa R, Leal M (2003) Thymic involvement in recovery of immunity among HIV-infected adults on highly active antiretroviral therapy. *J Antimicrob Chemother* 52(2):155–158. doi:10.1093/jac/dkg311
- De Martinis M, Franceschi C, Monti D et al (2006) Inflammation markers predicting frailty and mortality in the elderly. *Exp Mol Pathol* 80(3):219–227. doi:10.1016/j.yexmp.2005.11.004
- DelaRosa O, Pawelec G, Peralbo E et al (2006) Immunological biomarkers of ageing in man: changes in both innate and adaptive immunity are associated with health and longevity. *Biogerontology* 7(5-6):471–481. doi:10.1007/s10522-006-9062-6
- Douek DC, McFarland RD, Keiser PH et al (1998) Changes in thymic function with age and during the treatment of HIV infection. *Nature* 396:690–695. doi:10.1038/25374
- Franco JM, Rubio A, Martinez-Moya M et al (2002) T-cell repopulation and thymic volume in HIV-1-infected adult patients after highly active antiretroviral therapy. *Blood* 99(10):3702–3706. doi:10.1182/blood.V99.10.3702
- Girard D, Beaulieu AD (1997) Absence of the IL-7 receptor component CDw127 indicates that gamma(c) expression alone is insufficient for IL-7 to modulate human neutrophil responses. *Clin Immunol Immunopathol* 83(3):264–271. doi:10.1006/clin.1997.4341
- Goldberg GL, Zakrzewski JL, Perales MA et al (2007) Clinical strategies to enhance T cell reconstitution. *Semin Immunol* 19(5):289–296. doi:10.1016/j.smim.2007.08.001
- Goldberg GL, Alpodogan O, Muriglan SJ et al (2007b) Enhanced immune reconstitution by sex steroid ablation following allogeneic hemopoietic stem cell transplantation. *J Immunol* 178(11):7473–7484
- Gomez CR, Boehmer ED, Kovacs EJ et al (2005) The aging innate immune system. *Curr Opin Immunol* 17(5):457–462
- Gruver AL, Hudson LL, Sempowski GD (2007) Immunosenescence of ageing. *J Pathol* 211(2):144–156. doi:10.1002/path.2104
- Grzegorzewski K, Komschlies KL, Mori M et al (1994) Administration of recombinant human interleukin-7 to mice induces the exportation of myeloid progenitor cells from the bone marrow to peripheral sites. *Blood* 83(2):377–385

- Harris JM, Hazenberg MD, Pulin JF et al (2005) Multiparameter evaluation of human thymic function: interpretations and caveats. *Clin Immunol* 115(2):138–146. doi:[10.1016/j.clim.2004.12.008](https://doi.org/10.1016/j.clim.2004.12.008)
- Hazenberg MD, Otto SA, Cohen Stuart JW et al (2000) Increased cell division but not thymic dysfunction rapidly affects the T-cell receptor excision circle content of the naïve T cell population in HIV-1 infection. *Nat Med* 6(9):1036–1042. doi:[10.1038/79549](https://doi.org/10.1038/79549)
- Jamieson BD, Douek DC, Killian S et al (1999) Generation of functional thymocytes in the human adult. *Immunity* 10(5):569–575. doi:[10.1016/S1074-7613\(00\)80056-4](https://doi.org/10.1016/S1074-7613(00)80056-4)
- Jiang Q, Li WQ, Aiello FB et al (2005) Retroviral transduction of IL-7Ralpha into IL-7Ralpha-/- bone marrow progenitors: correction of lymphoid deficiency and induction of neutrophilia. *Gene Ther* 12(24):1761–1768. doi:[10.1038/sj.gt.3302558](https://doi.org/10.1038/sj.gt.3302558)
- Kilpatrick RD, Rickabaugh T, Hultin LE et al (2008) Homeostasis of the naïve CD4+ T cell compartment during aging. *J Immunol* 180(3):1499–1507
- Kumamoto T, Inaba M, Toki J et al (1995) Cytotoxic effects of irradiation and deoxyguanosine on fetal thymus. *Immunobiology* 192(5):365–381
- Lord JM, Butcher S, Killampali V et al (2001) Neutrophil ageing and immunesenescence. *Mech Ageing Dev* 122(14):1521–1535. doi:[10.1016/S0047-6374\(01\)00285-8](https://doi.org/10.1016/S0047-6374(01)00285-8)
- Mackall CL, Fleisher TA, Brown MR et al (1995) Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. *N Engl J Med* 332(3):143–149. doi:[10.1056/NEJM199501193320303](https://doi.org/10.1056/NEJM199501193320303)
- Marinova TT (2005) Epithelial framework reorganization during human thymus involution. *Gerontology* 51(1):14–18. doi:[10.1159/000081429](https://doi.org/10.1159/000081429)
- Marusic M, Turkalj-Kljajic M, Petroveckii M et al (1998) Indirect demonstration of the lifetime function of human thymus. *Clin Exp Immunol* 111(2):450–456. doi:[10.1046/j.1365-2249.1998.00470.x](https://doi.org/10.1046/j.1365-2249.1998.00470.x)
- McCune JM, Loftus R, Schmidt DK et al (1998) High prevalence of thymic tissue in adults with human immunodeficiency virus-1 infection. *J Clin Invest* 101(11):2299–2300. doi:[10.1172/JCI2834](https://doi.org/10.1172/JCI2834)
- Pawelec G, Koch S, Gouttefangeas C et al (2006) Immunorejuvenation in the elderly. *Rejuvenation Res* 9(1):111–116. doi:[10.1089/rej.2006.9.111](https://doi.org/10.1089/rej.2006.9.111)
- Poulin JF, Viswathan MN, Harris JM et al (1999) Direct evidence for thymic function in adult humans. *J Exp Med* 190(4):479–486. doi:[10.1084/jem.190.4.479](https://doi.org/10.1084/jem.190.4.479)
- Sempowski GD, Hale LP, Sundy JS et al (2000) Leukemia inhibitory factor, oncostatin M, IL-6, and stem cell factor mRNA expression in human thymus increases with age and is associated with thymic atrophy. *J Immunol* 164(4):2180–2187
- Sempowski GD, Gooding ME, Liao HX et al (2002) T cell receptor excision circle assessment of thymopoiesis in aging mice. *Mol Immunol* 38(11):841–848. doi:[10.1016/S0161-5890\(01\)00122-5](https://doi.org/10.1016/S0161-5890(01)00122-5)
- Shanker A (2004) Is thymus redundant after adulthood? *Immunol Lett* 91(2-3):79–86. doi:[10.1016/j.imlet.2003.12.012](https://doi.org/10.1016/j.imlet.2003.12.012)
- Strindhall J, Nilsson BO, Lofgren S et al (2007) No Immune Risk Profile among individuals who reach 100 years of age: findings from the Swedish NONA immune longitudinal study. *Exp Gerontol* 42(8):753–761. doi:[10.1016/j.exger.2007.05.001](https://doi.org/10.1016/j.exger.2007.05.001)
- Sutherland JS, Goldberg GL, Hammett MV et al (2005) Activation of thymic regeneration in mice and humans following androgen blockade. *J Immunol* 175(4):2741–2753
- Targonski PV, Jacobson RM, Poland GA (2007) Immunosenescence: role and measurement in influenza vaccine response among the elderly. *Vaccine* 25(16):3066–3069. doi:[10.1016/j.vaccine.2007.01.025](https://doi.org/10.1016/j.vaccine.2007.01.025)
- Yoshikawa TT (2000) Epidemiology and unique aspects of aging and infectious diseases. *Clin Infect Dis* 30(6):931–933. doi:[10.1086/313792](https://doi.org/10.1086/313792)