**Title:** Thymectomy in early childhood: significant alterations of the CD4^+CD45RA^+CD62L^+ T cell compartment in later life

**Running title:** Thymectomy in early childhood

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The study was aimed to assess indicators of immunosenescence, such as the total counts of peripheral blood CD4⁺CD45RA⁺CD62L⁺ (naive) T cells, the numbers of T cell receptor excision circles (TRECs), and Ki67-expression as marker of peripheral replication in thymectomized patients (TP) (n=101) compared to age-matched healthy donors (HD) (n=81). In TP, there was an inverse correlation between naive T cells and chronological age (p<0.001) or time post thymectomy (p<0.001). TP demonstrated lower TREC numbers in naive T cells compared to HD (p<0.001). TREC numbers negatively correlated with time post thymectomy (p<0.001). Percentages of Ki67-expresssing naive T cells were higher in TP compared to HD (p<0.05).

The findings of the presented long-term follow up cohort of thymectomized patients indicate that changes of the peripheral naive T cell subset in TP may resemble the findings of an aging immune system in elderly persons after thymic involution. Our data provide evidence that peripheral T cell homeostasis in TP is maintained at minimal levels mainly by extrathymic expansion of existing naive T cells in the periphery to compensate the diminished thymic output.

**Keywords:** thymectomy, naive T cells, aging, children, cardiac surgery, TREC, replication, immunosenescence
INTRODUCTION

The thymus, which is the primary site of T cell lymphopoiesis during fetal and early postnatal life, is almost fully developed at birth, but its involution continues throughout life [1, 2]. In the elderly, there is still ongoing T cell receptor gene rearrangement and T cell maturation, but a diminished thymic output of newly generated T cells and compensatory extrathymic expansion of existing naive T cells in the periphery [1-3]. Decrease of naive T cells which are characterized by the CD45RA isoform along with expression of the lymph node homing receptor CD62L [4] has been discussed to be responsible for the increased occurrence and severity of infections and autoimmune diseases in old age. Another most widely acknowledged phenotypic change of the aged individual is the loss of the major co-stimulatory molecule CD28 [3].

In open heart surgery for congenital heart diseases in children, the thymus is removed for better surgical access to the heart and great vessels. The thymus of children is highly active in the first months of life. Experiments in mice, discovering the importance of the thymus in early phases of life demonstrated that thymectomy resulted in partial immunodeficiency, mainly affecting cell mediated immune responses [5]. Thymectomy in children younger than 6 months has been shown to be tolerated without clinical consequences such as higher infection rates [6,7], but revealed to cause alterations of the peripheral T cell compartment. Changes in T cell phenotype following thymectomy were also described by several other studies, which reported decreased CD4+ T cell numbers [8-12].

However, there are no phenotypic markers that distinguish recent thymic emigrants from naive T cells produced by peripheral clonal expansion. The evaluation of T cell receptor recombination excision circle (TREC) numbers in peripheral T cells can be a useful indicator of thymopoiesis [13-16]. TRECs are not replicated during mitosis and, therefore, diluted out during cell divisions, which not only include priming of recent thymic emigrants to become
memory T cells but also homeostatic cell division of naive T cells. Children with primary and secondary immune deficiency diseases including human immunodeficiency virus (HIV)-infected children and patients with absent thymus, such as in severe combined immune deficiency disease or in complete DiGeorge syndrome showed significant decreased TREC levels [17-19]. TREC levels were markedly reduced in thymectomized patients concurrently with decreases in total T cells and phenotypic naive T-cells [9,20].

However, all these studies reported short-term surveys and did not consider long-term effects of thymectomy on the naive T cell subset which shows the most striking changes in the elderly after physiological thymic involution. Therefore, we examined thymic function and alterations to the peripheral naive T cell compartment in a cohort of patients who had undergone thymectomy in their first months of life for signs of premature immunosenescence in later life with the oldest patients having thymectomy more than 27 years ago. Premature immunosenescence after thymectomy may be of clinical relevance with advancing age of individuals regarding increased incidence of diseases of the elderly, such as infectious complications, artherosclerosis, neurodegenerative disorders and malignancies, and poor responses to new antigens, such as vaccine antigens [3]. To evaluate whether thymectomized patients demonstrate changes of the naive T cell pool with alterations reminiscent of the ones described in the elderly, three indicators of aging were measured: (1) the total counts of peripheral blood CD4⁺CD45RA⁺CD62L⁺ (naive) T cells; (2) the numbers of TREC in CD4⁺CD45RA⁺CD62L⁺ T cells; (3) Ki67-expression as marker of replicative history and proliferation.
MATERIALS AND METHODS

Study population. Peripheral blood mononuclear cells (PBMCs) were obtained from 101 thymectomized patients (TP) who had undergone thymectomy during open heart surgery for congenital heart disease and 81 age-matched healthy donors (HD) (table 1). Although differences in the mean and median chronological ages of TP (mean 11.1 ± 7.8 years) and HD (mean 14.1 ± 8.2 years) were statistically not significant, standard deviations were large. Therefore, we categorized the results from TP and HD into two age groups (≤12 years and >12 years of chronological age).

Thymectomy was performed by total resection of both lobes for ease of surgical access to the heart and major vessels, and not for other reasons. By the application of this surgical technique, in situ remains of residual cervical extensions of thymic tissue could not be excluded. Generally, these thymic tissue remnants were either macroscopically not visible or only a few millimetres in size. Regeneration of thymic tissue was excluded by thoracic magnetic resonance tomography or computer tomography in five cases and by mediastinal sonography in one case. In all cases, recent thoracic X-ray did not show any signs of remaining thymic tissue. Re-operation in seven cases did not reveal macroscopic thymic tissue. We included only TP, whose health was not impaired due to their heart condition. Exclusion criteria were residual cyanosis, transplantation or immunosuppressive therapy, cortisone therapy or hematologic disorders, medication with drugs known to influence blood production in the bone marrow or the immune system, allergic disorders, syndromes (e.g. Down syndrome, excluded by genetic screening for trisomy 21, or DiGeorge syndrome, excluded by genetic screening for 22q11 deletion and clinical examination), vaccination or infections in the last two to six weeks prior to taking the blood sample. Furthermore, there were no records of any blood transfusions, or further early childhood illness after surgical treatment of the heart defect for all included TP. The study was conducted in accordance with
Good Clinical Practice (ICH-GCP), the Declaration of Helsinki 2000, and local rules and regulations of the country. The study was approved by the local Ethics Committee, Medical University Innsbruck. All participants gave their written informed consent.

**Quantification of T cell subsets.** PBMCs were incubated with monoclonal mouse antibodies (mAbs) specific for CD3, CD4, CD28, CD45RA, CD45RO, and CD62L labeled with FITC, PE, PerCP or APC (all antibodies purchased from BD Pharmingen, San Jose, CA, USA) for 20 min at room temperature in the dark. After incubation, red blood cell lysis was performed with FACS-Lysing-solution (BD Pharmingen). Subsequently, cells were washed twice with PBS and fixed with 2% paraformaldehyde. All analyses were performed using a FACS Calibur flow cytometer (Becton Dickinson, Oxford, United Kingdom) utilizing CELLQuest software (BD Pharmingen). A minimum of 3,000 events was counted for each panel in FACS analysis. Results were expressed as total counts or percentages of gated lymphocytes. According to phenotypic CD markers, CD4⁺CD28⁺CD45RA⁺ and CD4⁺CD45RA⁺CD62L⁺ T cells were characterized as naive and CD4⁺CD28⁺CD45RO⁺ as early memory T cells in children and young adults [21]. CD28 was chosen as an additional marker for CD45RA⁺ naive T cells to allow discrimination between naive T cells and CD45RA⁺CD62L⁺ but CD28⁻ effector T cells.

**Separation of T cell subsets.** PBMCs were isolated by using LymphotoPrep™ (Axis Shield, Oslo, Norway) according to manufacturer’s instructions. CD4⁺CD45RA⁺CD62L⁺ (naive) T cells were separated from 26 TP and 29 HD by using an Auto MACS system with sterile columns (Miltenyi Biotec, Teterow, Germany). CD4⁺CD45RA⁺ T cells were collected by using a naive CD4⁺ T cell isolation kit for negative selection (Miltenyi Biotec). Subsequently, CD4⁺CD45RA⁺ T cells were incubated with mouse-anti-human CD62L antibodies (isotype IgG2a, BD Pharmingen) and labeled indirectly with rat-anti-mouse IgG2a magnetic
microbeads (Miltenyi Biotec) for positive selection by Auto MACS. Purity of separated CD4⁺CD45RA⁺CD62L⁺ T cells was checked using flow cytometry (FACS-Calibur flow cytometer) and ranged from 97 to 99%.

**Quantification of TREC numbers.** DNA was extracted from separated CD4⁺CD45RA⁺CD62L⁺ T cells using QIAamp DNA Mini Kit (Qiagen, Chatsworth, CA, USA). In order to remove contaminations, which would interfere with polymerase chain reaction (PCR), DNA was purified by ethanol-precipitation using 0.4M LiCl₂ and 2.5-fold the volume 100% ethanol at –20°C for 30 min. After centrifugation, the pellet was washed twice with 70% ethanol to remove remaining salts. The pellet was dissolved in nuclease-free water. Signal-joint TREC concentrations were determined by quantitative SYBR-green real-time PCR based on the coding TREC sequence using an iCycler quantitative RT-PCR system (BioRad Laboratories, Hercules, Canada). We designed primers to amplify a DNA fragment 82 bp across the remaining recombination sequence δrec/ψalpha (5’-CAC ATC CCT TTC AAC CAT GCT-3’ and 5’-GCC AGC TGC AGG GTT TAG G-3’). For quantification we used the internal standard as previously described [13]. PCR reaction was run with 0.5 µg DNA, primers and SYBR™ Green Supermix (Bio-Rad Laboratories, Hercules, Canada) in a final volume of 25 µl. Each experiment was performed in duplicates and log₂ dilutions of the internal standard were used to quantify the number of TRECs in each sample. To avoid bias by different numbers of T cells, TRECs were calculated in relation to CD4⁺CD45RA⁺CD62L⁺ T cell numbers [22]. However, interpretation of TREC numbers is limited by the fact that TREC numbers per naive T cells are also diluted by cell replication. Therefore, Ki67-expression was used as a marker for replication of CD4⁺CD45RA⁺CD62L⁺ T cells.

**Ki67 staining.** CD4⁺CD45RA⁺CD62L⁺ T cells in the cell cycle were identified by expression of the Ki67 nuclear antigen. Determination of Ki67-expression on CD4⁺CD45RA⁺CD62L⁺ T
cells was performed by cytospin preparation (Shandon cytospin 4, Waltham, MA, USA) of $5 \times 10^3$ CD4$^+$CD45RA$^+$CD62L$^+$ T cells. After fixation with 2% paraformaldehyde, cells were permeabilized with 0.05% Triton X-100/0.1% Sodium-citrate on the object slides (Sigma, St. Louis, MO, USA) and washed twice. Cells were stained with mouse-anti-human-Ki67 antibody (MIB-1 clone; DAKO, Glostrup, Denmark) and secondly, with goat-anti-mouse-peroxidase (-POD) monoclonal antibody (DAKO). The second antibody was developed with 3,3-diaminobenzidine tetrahydrochloride (DAB) (iVIEW DAB Detection, Ventana Medical Systems, Tucson, AZ, USA). Immunohistochemistry was undertaken in a fully automated Nexes system (Ventana Medical Systems). For microscopic orientation counterstaining was performed with Hematoxylin Counterstain and Bluing Reagent (Ventana Medical Systems). Stained cells were examined by two independent investigators (MP and MZ) by light microscopy (Eclipse 800, Nikon). Percentages of Ki67$^+$ cells were calculated per total CD4$^+$CD45RA$^+$CD62L$^+$ T-cell counts on each cytospin preparation.

**Statistical analysis.** Data were tested for normal distribution by a Kolmogorov-Smirnov test. For two independent groups of variables that did not follow a normal distribution, the non-parametric Mann-Whitney-U test was applied. For more than two independent groups of variables that did not follow a normal distribution, the non-parametric Kruskal-Wallis-test was applied to avoid multiple testing bias. For testing the correlation between different variables, the Spearman's rank correlation coefficient was calculated. A generalized linear model was used to infer the influence of the time post thymectomy on the immunological system, adjusted for the chronological age of the patient. All statistical analyses were performed with SPSS 15.0 (SPSS Inc., Chicago, IL, USA).
RESULTS

Clinical data. None of the TP or HD had suffered from autoimmune diseases or malignancies. None of the TP and HD had measurable anti-nuclear-antibodies or elevated levels of rheumatoid factor. Two TP reported severe infections in the first five years after thymectomy (one had pneumonia and one appendicitis). Three TP had chronic relapsing upper airway infections and sinusitis. Three TP had a documented urinary tract infection without kidney involvement. In the HD group no severe or chronic infections were reported. One HD had an urinary tract infection and one had bronchitis. There was no case of asthma, candidiasis or allergy in either group.

Reduction of naive T cell numbers with chronological age and time post thymectomy in TP. Initially, we compared the total counts (table 2) of peripheral CD4⁺ T cells expressing naive (CD45RA⁺CD62L⁺) and memory (CD28⁺45RO⁺) surface markers in TP and HD. TP (>12 years) showed lower total counts of CD4⁺ (p<0.001), CD4⁺CD28⁺CD45RA⁺ (p<0.001) and CD4⁺CD45RA⁺CD62L⁺ (p<0.01) than age-matched HD (>12 years) (table 2). In both, TP (> 12 years) and HD (>12 years) CD4⁺ naive T cells were lower compared to individuals ≤12 years of age (CD4⁺CD28⁺CD45RA⁺: p<0.001; CD4⁺CD45RA⁺CD62L⁺: p<0.01).

Although of high individual variability and limited by a cross-sectional study design, trends for lower CD4⁺ naive T cells with chronological age (p<0.001) (figure 1A and B, 2A and B) compared to HD and time post thymectomy (p<0.001) (figure 3; table 3) may be described. Total counts of CD4⁺CD28⁺CD45RO⁺ T cells positively correlated with chronological age (p<0.001) and time post thymectomy (p<0.001) (table 3).

Reduction of TREC numbers in CD4⁺CD45RA⁺CD62L⁺ T cells of TP. Thymic activity and output of recent thymic emigrants was assessed by measuring numbers of TREC in peripheral CD4⁺CD45RA⁺CD62L⁺ T cells. Performing CD4⁺CD45RA⁺CD62L⁺
quantification and TREC analysis was only possible in individuals in whom blood sample size was large enough to separate at least 10000 CD4⁺CD45RA⁺CD62L⁺ for DNA preparation. For this reason, data of some small children as well of some older patients are missing due to technical limitations. Although CD4⁺CD45RA⁺CD62L⁺ counts were higher in younger children (table 2), only small blood volumes were available. On the other hand, CD4⁺CD45RA⁺CD62L⁺ counts in older patients were approximately three times lower than in younger children (table 3). Therefore, separation of this T cell subset was not possible in all cases. However, a phenotypic difference between TP (>12 years) included into CD62L, TREC and Ki67 evaluation [n=24] and age-matched TP (>12 years) not included [n=19] was excluded by statistical analysis of CD4⁺CD28⁺CD45RA⁺ T cell subsets within the groups (data not shown).

In HD and TP, there was a significant inverse correlation between chronological age and TREC in the peripheral CD4⁺CD45RA⁺CD62L⁺ T cell pool (p<0.001 for HD and TP), reflecting the physiological decrease in thymic function with chronological age (figure 4A; table 3). TP (>12 years) showed 3.6 times lower TREC than HD (>12 years) (p<0.001) (table 2). TREC were positively correlated with time post thymectomy (p<0.001) (figure 4B; table 3).

**Higher Ki67-expression in CD4⁺CD45RA⁺CD62L⁺ T cells of TP.** TREC concentrations are influenced by two parameters, the output of newly generated thymic T cells and the dilution of TREC-positive T cells through replication of peripheral T cells. The reduction of TREC could indicate diminished thymic T cell production of thymectomized individuals. Increased compensatory peripheral naive T cell expansion could also contribute to the age-inappropriate decline of TREC in naive T cells. In either case, CD4⁺ T cells of TP should have undergone increased replication. To provide an estimate of the replicative activity, proliferative rates of CD4⁺CD45RA⁺CD62L⁺ T cells were estimated by measuring the fraction of Ki67-expressing
cells. TP (>12 years) had a significantly higher proportion of CD4+CD45RA+CD62L+ T cells in the cell cycle than HD (>12 years) (p<0.05) (table 2). In TP, percentages of Ki67+ CD4+CD45RA+CD62L+ T cells correlated with chronological age (p<0.001) and time post thymectomy (p<0.001) (figure 5A and B; table 3); the correlation with chronological age in HD was also significant (p<0.01).

**T cell subsets, TREC numbers, or Ki67-expression and time parameters.** To elucidate the role of time post thymectomy on T cell subsets, TREC numbers and Ki67-expression a generalized linear model with adjustment for the chronological age was used, as the chronological age significantly correlated with the time post thymectomy (correlation coefficient 0.878; p<0.001). CD4+CD28+CD45RA+ and CD4+CD28+CD45RO+ T cells were mainly influenced by chronological age (p<0.001 and p<0.01, respectively) (table 4). However, lower CD4+CD45RA+CD62L+ T cell counts and diminished TREC numbers were affected by both factors, chronological age (p<0.01 and p<0.05, respectively) and time post thymectomy (p<0.05 and p<0.05, respectively) (table 4).

There was a significant inverse correlation between TREC numbers and Ki67-expressing CD4+CD45RA+CD62L+ T cells in TP (correlation coefficient -0.840; p<0.001) and HD (correlation coefficient -0.721; p<0.001). Individuals with low CD4+CD45RA+CD62L+ T cell counts showed higher proportions of Ki67-expressing T cells (TP: correlation coefficient -0.710; p<0.001; HD: correlation coefficient -0.569; p<0.01).
DISCUSSION

Our findings provide evidence that thymectomy in early childhood results in significant alterations of the peripheral CD4⁺CD45RA⁺CD62L⁺ naive T cell subset which are reminiscent of loss of ongoing thymopoiesis in later life. The data of the long-term follow up cohort indicate that changes of the peripheral naive T cell subset in TP may resemble the findings of an aging immune system in elderly persons after thymic involution which is characterized by the loss of naive T cells [3,23,24]. Postnatal contribution of the thymus to immune development is still a matter of debate. However, recent studies in normal individuals have confirmed ongoing contribution of the thymus to the maintenance of the T cell compartment throughout life in old age [9,25-28].

Several studies have discussed the resulting phenotypic changes of the human immune system after childhood thymectomy due to cardiac surgery. These studies showed lower numbers of CD4⁺ T cells [10-12] and naive T cells [9,10] in the thymectomized group. They speculated that thymectomy was incomplete [11], T cells may be generated de novo at extrathymic sites [10,29], or that recent thymic emigrants generated before thymectomy persist for decades [30,31]. Investigations in a previously thymectomized patient who was irradiated before bone marrow transplantation [32] and a thymectomized AIDS patient [30] demonstrated the thymus dependence of CD4⁺ naive T cell generation and the presence of a long-lived CD4⁺CD45RA⁺CD62L⁺TREC⁻negative population of T cells. In accordance to these findings, in our study, the thymus-depleted immune system was capable to maintain CD4⁺ naive T cell counts, although to a lower level than age-matched HD, probably by increased replication of post-thymic naive T cells. Although continued production of recent thymic emigrants by retained small portions of the thymus could not entirely be excluded, the increased proportion of Ki67-expressing naive T cells suggests a peripheral homeostatic proliferation to restore the naive T cell pool. This theory is supported by a study in aged individuals which showed that
homeostatic proliferation occurring at a frequency of 0.1 – 0.4% of all naive T cells appeared to be the major source of T cell generation during adult life [33]. Intense scientific interest is focused on the exploration of extrathymically derived T cells. In a recent study, T cells produced on extrathymic sites revealed their undue susceptibility to apoptosis [34] and should explain the increase in Ki67-expressing replicating naive T cells to maintain a steady pool.

The hypothesis of thymus-dependent naive T cell production [30,32] is also supported by the fact that TREC numbers were diminished in the CD4+ naive T cells of TP and correlated with chronological age and time post thymectomy. However, several biologic parameters could complicate the interpretation of TREC data. TREC concentrations are therefore as much influenced by peripheral turnover as by the influx of new TREC-positive T cells from the thymus [35-37]. Therefore, TREC numbers are not only an estimate of thymus function but can be diluted by peripheral T cell proliferation. Following this assumption, our data are in accordance with a mathematical model for TREC kinetics after adult thymectomy, showing an accelerated decline in TREC numbers immediately after thymectomy but a relatively mild decrease of CD4+ naive T cells with advancing age [38]. Decreased thymic output may induce compensatory autoproliferation of peripheral naive T cells, a process that contributes to TREC dilution. Therefore, the data can be explained by a history of increased turnover in the naive T cell compartment, demonstrated by increased proportions of Ki67-expressing naive T cells. This may also be reflected by the significant influence of time post thymectomy on TREC numbers and Ki67-expressing naive T cells. However, the study at hand is a cross-sectional analysis aimed at assessing indicators of immunosenesence in thymectomized patients compared to age-matched healthy donors.

The design of the study allowed for the estimation of decrease parameters such as the total counts of peripheral blood CD4+CD45RA+CD62L+ T cells and the numbers of TREC's compared to normal controls rather than appraising exact decrease rates.
Another limitation of the study is given by the conflicting opinions which cell surface proteins are reliable markers of truly naive T cells. CD28 in addition to CD45RA serves as a marker to identify naive T cells predominately in young persons. Although a CD4^+CD28^+CD45RA^+ phenotype is generally considered as defining naive T cells, this definition may not be applied in elderly persons [39]. CD62L, CCR7 and CD27 have been also suggested to characterize naive T cells [40]. Therefore, we used CD62L to separate CD4^+ naive T cells. However, CD31 has been identified as an additional marker to distinguish between TREC enriched recent thymic emigrants co-expressing CD45RA and CD31 and peripherally expanded naive CD4^+ T cells which are characterized by the loss of CD31 expression and highly reduced TREC contents [41]. In future studies, correlation of TREC, Ki67-expressing CD4^+ naive T cells and levels of circulating interleukin-7 (IL-7) will provide a more detailed insight in peripheral T cell pool regulation in childhood and adolescence, as IL-7 was described as one of the most important cytokines regulating T cell development [42].

Taken together, our long-term follow up data indicate that thymectomy in infants and young children can result in long-term reduction of new T cell production as evidenced by a reduction in TREC number years later. Despite a reduction in thymic output of naive T cells and reduced peripheral CD4^+ T cells, naive T cells are maintained at minimal levels in thymectomized children. These data are supported by a study with previously thymectomized patients who had undergone cardiac transplantation [43]. In that study, remarkably low TREC numbers were found in total leukocytes of the patients with almost normal CD4^+ T cell numbers, thus suggesting a restoring of the T cell compartment by proliferation of residual peripheral T cells.

The question for long-term clinical consequences such as manifest immunodeficiency with increased infection rates or signs of autoimmunity as seen more frequently in persons after thymus involution is still unanswered. It could be speculated that the increased susceptibility of some elderly individuals to new pathogens and higher infection rates may be related to...
their inability to produce new CD4⁺CD45RA⁺ T cells [3]. The hypothesis that a diminished capacity to produce new thymic T cells and that an altered naive T cell homeostasis after childhood thymectomy may have a clinical impact on responses to new antigens was supported by our recent study showing a delayed antibody response to tick-borne encephalitis vaccination in thymectomized children [44]. However, it remains to be investigated whether thymectomized individuals will more rapidly lose their ability to generate new T cells and whether thymectomy in their early life creates an “immune-risk phenotype” [45,46] for disorders of the elderly in later life.

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REFERENCES


11. W.J. Wells, R. Parkman, E. Smorgorzewska, M. Barr, Neonatal thymectomy: does it
Helgason, H.M. Ogmundsdottir, The influence of partial or total thymectomy during
Picker, R.A. Koup, Changes in thymic function with age and during the treatment of
14. F. Kong, C.H. Chen, M.D. Cooper, Thymic function can be accurately monitored by
the level of recent T cell emigrants in the circulation, Immunity 8 (1998) 97-104.
15. C.M. Steffens, L. Al-Harthi, S. Shott, R. Yoge, A. Landay, Evaluation of
thymopoiesis using T cell receptor excision circles (TRECs): differential correlation
95-101.
16. P. Ye, D.E. Kirschner, Reevaluation of T cell receptor excision circles as a measure of
17. S. Chavan, B. Bennuri, M. Kharbanda, A. Chandrasekaran, S. Bakshi, S. Pahwa,
Evaluation of T-cell receptor gene rearrangement excision circles after antiretroviral
therapy in children infected with human immuno-deficiency virus, J. Infect. Dis. 183
Sempowski, M.E. Rhein, P. Szabolcs, L.P. Hale, R.H. Buckley, K.E. Coyne, H.E.
Rice, S.M. Mahaffey, M.A. Skinner, Complete DiGeorge syndrome: development of


LEGENDS TO FIGURES

Figure 1. Correlations of total counts of CD4^+CD28^+CD45RA^+ (A) and CD4^+CD45RA^-CD62L^- naive T cells (B) with chronological age.

Lines represent the theoretical fit of the experimental data by assuming an exponential regression model. R^2 reflects the proportion of variability that is accounted for by the non-linear regression model. TP: thymectomized patients; HD: healthy donors.

Figure 2. CD4^+CD45RA^-CD62L^- naive T cell subset distributions in healthy donors (HD) (A) and thymectomized patients (TP) (B).

The age-dependent distribution of these T cell subset is demonstrated by showing a representative example of one young TP and one age-matched young HD compared to one older TP and one older age-matched HD.

Figure 3. Correlations of total counts of CD4^+CD28^+CD45RA^+ (A) and CD4^+CD45RA^-CD62L^- naive T cells (B) with time post thymectomy.

Lines represent the theoretical fit of the experimental data by assuming an exponential regression model. R^2 reflects the proportion of variability that is accounted for by the non-linear regression model. TP: thymectomized patients; HD: healthy donors.

Figure 4. Correlations of TREC numbers with chronological age (A) or time post thymectomy (B).

Lines represent the theoretical fit of the experimental data by assuming an exponential regression model. R^2 reflects the proportion of variability that is accounted for by the non-linear regression model. TP: thymectomized patients; HD: healthy donors.
Figure 5. Correlations of percentages of Ki67⁺ CD4⁺CD45RA⁺CD62L⁺ naive T cells with
chronological age (A) and time post thymectomy (B).

Lines represent the theoretical fit of the experimental data by assuming an exponential
regression model. R² reflects the proportion of variability that is accounted for by the non-
linear regression model. TP: thymectomized patients; HD: healthy donors.