Levels of Recent Thymic Emigrant Cells Decrease in Children Undergoing Partial Thymectomy during Cardiac Surgery

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The human thymus is required for establishment of a T-cell pool in fetal life, but postnatal thymectomy is not known to cause immunodeficiency. T-cell emigration from thymus (thymic recent emigrants [TRECs]) is a continuous thymic-dependent process. We studied TREC levels pre- and post-partial-thymectomy in children undergoing cardiac surgery. TRECs were quantitated by real-time PCR in peripheral blood lymphocytes of 24 children (0 to 12 years). TREC values were 47916 ± 9271 pre-partial thymectomy and 33157 ± 8479 post-partial thymectomy in 11 paired patients (P = 0.014). Interval between pre- and post-partial thymectomy was 8.8 days ± 5.8 days. Another group of 8 children had 30384 ± 9748 TRECs 16 days to 6 years post-partial thymectomy. There was a significant drop in TREC values post-partial thymectomy in the immediate postoperative period compared to prethymectomy TREC levels. While decreased thymic output may persist, the long-term implications were not evaluated in this patient population.

The thymus plays a crucial role in the development of T cells providing an inductive microenvironment in which bone marrow-derived progenitors undergo proliferation, T-cell receptor gene rearrangement and thymocyte differentiation into mature T cells.

Two types of cells are generated in the thymus. Most express a T-cell receptor composed of α and β chains, but a small population expresses a receptor of γ and δ chains. All of these chains are encoded by variable (V), diversity (D), and junctional (J) gene segments, which are rearranged during T-cell development in a process called V(D)J recombination. This process involves cleavage of DNA at the recombination signal sequences that flank T-cell receptor gene segments in their germ line configuration. When the intervening stretches of DNA are excised, then the coding ends are joined to form a functional T-cell receptor gene in the chromosomal DNA, and the signal ends join to form extra chromosomal DNA circles termed T-cell receptor rearrangement excision circles (TRECs). The T-cell receptor-δ locus is embedded in the T-cell receptor-α locus; so T-cell receptor-δ sequences are specifically deleted in all αβ T cells. During T-cell receptor rearrangement there are two rearrangement events which happen, first producing a signal joint TREC and second producing a coding joint TREC (11).

In humans there is no known way to distinguish phenotypically between cells that have recently emigrated the thymus and long-lived naïve cells in the periphery. One proposed marker for recent thymic emigrants is the episomal DNA circle that is generated during excision rearrangement of T-cell receptor genes. TRECs are stable and are not duplicated during mitosis (3). Signal and coding joint TRECs are affected similarly in mature T cells.

Children with congenital heart diseases routinely undergo partial thymectomy during cardiac surgery for better visualization of structures. There have been few studies done to determine the effects of neonatal thymectomy in humans (1, 13). In the report focused on neonatal thymectomy in humans, Brearley et al. (1) had shown that in infants younger than 3 months of age thymectomy caused impaired immunity in late childhood and concluded that thymectomy in pediatric cardiac surgery should be avoided. Wells et al. (13) did not concur on this concept of preserving the thymus and showed that total number of T cells and CD4 cells dropped 12 months after thymectomy.

In chickens (7) levels of chT1+ cells (which correlated to TRECs) disappeared after complete thymectomy. In chickens with partial thymectomies the levels of chT1+ T cells in the circulation 4 weeks postthymectomy correlated directly with the numbers of residual thymic lobes.

There have been no studies done to determine what happens to TREC levels after partial thymectomy in children with congenital heart disease. This study was done to study the effect of partial thymectomy on TREC values. Partial thymectomy is defined as removal of 70 to 90% of thymic gland from the anterior mediastinum to facilitate exposure for cannulation for cardiopulmonary bypass.

MATERIALS AND METHODS

The institutional ethical and research review boards approved the study and informed consent was obtained from the parents. Twenty-four children (age range, 0 to 12 years, mean = 4 ± 3.8 years) with congenital heart disease were prospectively entered into the study. They were subdivided into three groups. Group A included patients undergoing first surgery for congenital heart disease. Both pre- and post-partial thymectomy samples were collected after 3 to 15 days from 11 patients.

Group B included patients undergoing reoperation for congenital heart dis-
ease. (Only post-partial thymectomy samples were collected from 8 patients, after an interval of 16 days to 6 years.)

Group C included patients with congenital heart disease undergoing cardiac catheterization. (Only pre-partial thymectomy samples were collected from 5 patients.)

No study patient carried the diagnosis of DiGeorge syndrome. No patients received steroids during the hospitalization.

Cell sources. Peripheral blood samples were drawn from patients. Three milliliters of whole blood was collected in sterile heparinized tubes processed within 24 h.

Cell isolation and quantification of TRECs by real-time PCR. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll density gradient centrifugation. Cells lysates were prepared by proteinase K digestion TRECs were quantified by real-time PCR analysis, using the TaqMan 5 nuclease assay, and the ABI Prism 7700 sequence detection system (PE-Applied Biosystems). In brief, 0.5 μM primers (forward primer: CACATCCCTTTCAACCATGCT and reverse primer: GCCAGCTGAGGTTTAGG), 0.1 μM TaqMan probe (FAM-ACACCTCTGTTTTTGTAAAGGTGCCCACT-TAMRA), 3.5 mM MgCl₂, 0.2 mM deoxynucleoside triphosphates, 0.25 U of uracil DNA glycosylase, 0.625 U of AmpliTaq Gold DNA polymerase in TaqMan buffer (PE Biosystem), and 100.250 ng of genomic DNA from PBMC were mixed together in a 25-L PCR mixture. PCR conditions were 50°C for 2 min, 95°C for 10 min, 95°C for 30 s, and 60°C for 1 min for 45 cycles. A standard curve was established with known copies of plasmids containing signal joint TRE fragment and TRE values for test samples were calculated with the software provided with the ABI Prism 7700 system (Sequence Detection System, version 1.6.3). Each sample was run in duplicate and mean TREC values were used for data analysis. Results were expressed as TRECs/10⁶ cells.

Statistical analysis. The significance of differences between means in TREC values was determined by use of the Student t test, Wilcoxon signed rank sum test, and Mann-Whitney U test.

RESULTS

TREC values were recorded per million peripheral blood mononuclear cells. TREC levels, group A: for patients from group A (n = 11), the average interval between the samples was 8.8 days ± 5.8 days. The pre-partial thymectomy TREC value was 47,916 ± 30,748, (mean ± standard error of mean) with the post-partial thymectomy TREC value being 33,157 ± 28,122, (mean ± standard error of mean) (Fig. 1). This decrease in the post-partial thymectomy TREC value was significant (P = 0.014, Wilcoxon signed rank sum test).

TREC levels in patients in group B (n = 8) were 30,384 ± 9,748 (mean ± standard error of mean).

TREC levels in patients in group C (n = 5) were 69,774 ± 33,601 (mean ± standard error of mean).

Although no statistical correlation was drawn between groups B and C as they included different patients, the overall pre-partial thymectomy TREC levels were higher than the post-partial thymectomy TREC levels.

DISCUSSION

The thymus is the primary site of T-cell lymphopoiesis during fetal and early postnatal life. Studies of thymuses of patients with immune deficiencies have led to an understanding of the molecular and genetic basis of many immune deficiency diseases, pointing to the importance of the thymus in the development (12). Due to thymic involution, it has been suggested that the adult thymus does not contribute to the new T-cell replacement. However recent data indicate that the thymus contributes to new T cells even in adulthood (4, 6, 10). Thus, it is highly likely that a decrease in thymopoiesis would lead to a lasting deficiency in the ability of the host to generate new T cells. There are no phenotypic markers that distinguish between recent thymic emigrants from the rest of peripheral naive T-cell pool. The evaluation of TREC frequencies in peripheral T cells can be a useful indicator of the de novo production of T lymphocytes (3).

In children, studies using TREC’s have been performed in patients with primary and secondary immune deficiency diseases including human immunodeficiency virus (HIV)-infected infants and children (2, 8, 9). Patients with absent thymus, such as in severe combined immune deficiency disease or in complete DiGeorge syndrome have very low TREC’s. In HIV-infected infants, TREC’s in peripheral blood lymphocytes were decreased when compared to HIV-exposed, uninfected infants; after therapy the TREC levels increased to levels present in HIV-exposed, uninfected infants (2). In a recent study, TREC’s were markedly reduced in patients many years after thymectomy in childhood, concurrently with decreases in total T cells and phenotypic naive CD4 T cells (5).

In the present study the TREC levels significantly declined in all study patients post-partial thymectomy in the immediate postoperative period. The decreased TREC values seen in patients in the immediate postoperative period are probably due to several reasons, including decreased thymic output and changes in blood volume distributions. Overall the TREC values in patient groups without thymectomy were higher than the patients who had a partial thymectomy. The likelihood of a true decrease in TREC’s resulting from decreased thymopoiesis is strengthened by the observation that even patients examined for periods up to six years post-partial thymectomy...
had lower TRECs than patients who did not have thymectomy. In the published study of Halnon et al. (5), patients with no residual thymus had significantly lower TRECs than those with partial thymectomy. Whether the decrease in TREC in patients with partial or complete thymic removal also leads to a deficiency in thymic-derived regulatory T cells is unknown. The partial thymectomy in infancy and decrease in TRECs did not appear to adversely influence immune function, as there was no known autoimmune diseases in the study population over a short term follow up. Presumably, minimal residual thymic tissue post-partial thymectomy is sufficient for immune function.

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REFERENCES


