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ORIGINAL ARTICLE

Comparison of major lymphocyte subpopulations and recent thymic emigrants in patients with ataxia telangiectasia and age-matched healthy groups

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Abstract
Background: Ataxia telangiectasia (A-T) is a genetic disorder caused by the homozygous mutation of the A-T mutated gene. It is frequently associated with variable degrees of cellular and humoral immunodeficiency. However, the immune defects in A-T patients are not well characterized. To the best of our knowledge, no studies have focused on the major lymphocyte subpopulations and recent thymic emigrants of A-T patients in comparison with age-matched healthy controls.
Methods: Following the European Society for Immunodeficiencies criteria, 17 patients diagnosed with A-The, and 12 age-matched healthy children were assigned to the study. Both patients and healthy controls were grouped as 1–5, 6–10, 11–15, and 15+ years. By using a flow cytometer, major lymphocyte subpopulations and CD4+CD45RA+CD31+ recent thymic emigrants were determined as percentage and absolute cell numbers and compared.
Results: No significant differences in all lymphocyte subpopulations were observed between the age groups of A-T patients. Compared to the healthy controls, there was a decrease in T cells, effector memory T4 cells, B cells, naïve B cells, naïve T4 cells, switched B cells, and recent thymic emigrants and an increase in active T8 cells and non-switched B cells in the percentage and absolute number of some cell populations in the A-T group.
Conclusions: This study showed that effector functions in some cell lymphocyte populations were decreased in A-T patients.

Introduction

Ataxia telangiectasia is a genetic disorder caused by the homozygous mutation of the A-T mutated (ATM) gene. It is frequently associated with variable degrees of cellular and

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humoral immunodeficiency. Immunodeficiency affects over half of all A-T patients and can contribute significantly to morbidity and mortality.[1] However, the immune defects in A-T patients are not well characterized.[2,3]

To the best of our knowledge, no studies have focused on major lymphocyte subpopulations and recent thymic emigrants (RTE) of A-T patients in comparison with age-matched healthy controls. Therefore, this study aimed to clarify the relationship of age and major lymphocyte subpopulations and RTE of A-T patients that could have some effects on the clinical status of patients.

Patients and methods

Patients who were diagnosed with A-T at two medical centers – Ondokuz Mayıs University, Medical Faculty, Department of Pediatric Allergy and Immunology, and Inonu University Medical Faculty, Department of Pediatric Allergy and Immunology – between 2003 and 2013 were included in the study. A-T was diagnosed according to the European Society for Immunodeficiencies (ESID) criteria. Blood samples of these patients and age-matched healthy children were taken between August and November 2013. Both patients and healthy children were grouped as 1–5, 6–10, 11–15, and 15+ years. All blood samples were evaluated for absolute lymphocyte counts using an Abbot blood counter (Cell Dyn Emerald USA). By using flow cytometry, major lymphocyte subpopulations and CD4+CD45RA+CD31+ RTE were determined as percentage and absolute cell numbers and compared in both groups.

Financial support for the immunological evaluation of A-T patients was provided by governmental health insurance. For healthy children, the stock of the Ondokuz Mayis University's Pediatric Immunology-Allergy Diagnostic and Research Laboratory was used.

Evaluation of lymphocyte subpopulations by flow cytometry

A total of 3 cc peripheral venous blood was collected from each subject into ethylenediaminetetraacetic acid (EDTA) tubes. 100 μL blood was added to the tubes and then incubated with a 10 μL panel of conjugated antibodies CD45, CD3, CD4, CD8, CD19, CD45RA, CD31, CD56, HLA-DR, CD62L, CD27, and IgD (BD Biosciences, Heidelberg, Germany) for 20 min at room temperature away from light. Then, samples were lysed by incubating in FACS lysing solution for 15 min. Cells stained with isotypic controls for IgG1-FITC or PE were used as negative controls. After appropriate gating with lymphocytes, the lymphocyte populations were determined. The cytometer was set to acquire 10,000 events. Data were processed using CELL Quest software program (Becton Dickinson). These analyses were conducted for all subjects and were all performed by the same well-trained operator, who was unaware of the patients and healthy children. Based on the absolute lymphocyte counts, the absolute numbers of the lymphocyte subpopulations and RTE were proportioned as the number of cells per 1 mL of whole blood.

Statistical analysis

The results were expressed as median (min–max) values. Because the data of both groups did not show a normal distribution, Mann–Whitney *U* test and Kruskal–Wallis test were used to evaluate all the data. Correlation analysis was used to evaluate the relationship between the two groups.

Ethical disclosures

Ethics committee approval was granted by decision no. OMU KAEK 2013/331 dated 11.07.2013 by the Ondokuz Mayis University's Ethics Committee of Medical Research.

Results

Seventeen A-T patients (11 male, 6 female, median: 9, min–max: 3–20 years) and 12 age-matched healthy children (4 male, 8 female, median: 10, min–max: 2–18 years) were assigned to this study. Neurological manifestations (e.g., ataxia, dysmetria), dermatological manifestations (e.g., telangiectasia, hypopigmentation), and immunological manifestations (e.g., hypogammaglobulinemia, lymphopenia) were seen in 17 (100%), 16 (94%), and 14 (82%) patients, respectively. Table 1 shows the demographic data of patients with ataxia telangiectasia.

No significant differences regarding all lymphocyte subpopulations were observed between the age groups of A-T patients (Table 2). Compared to the healthy children, there was a decrease in the percentage and absolute number of some cell populations in the A-T group as follows: T cells (in 11–15 years group, $p = 0.023$), effector memory T4 cells (in 11–15 years group, $p = 0.08$), B cells (in 6–10 and 11–15 years groups, $p = 0.033$ and $p = 0.018$, respectively), naïve B cells (in 11–15 years group, $p = 0.023$), naïve T4 cells (in 6–10 years group, $p = 0.026$), switched B cells (in 6–10 years group, $p = 0.041$), and RTE (in 6–10 and 11–15 years group, $p = 0.013$ and $p = 0.010$, respectively).

Compared to the healthy children, there was an *increase* in the percentage and absolute number of some cell populations in the A-T group as follows: active T8 cells (in 11–15 years group, $p = 0.08$) and non-switched B cells (in 6–10 years group, $p = 0.041$) (Table 3).

Discussion

In this study, we evaluated the lymphocyte subpopulations and RTE of A-T patients and compared them with those of age-matched healthy children to understand whether there is any change or deterioration with age. There were no significant differences regarding all lymphocyte subpopulations between the age groups of A-T patients. However, compared to the healthy controls, there was a decrease in the percentage and absolute number of T cells, effector memory T4 cells, B cells, naïve B cells, naïve T4 cells, switched B cells, and RTE, and an increase in active T8 cells and non-switched B cells. The decreased effector and thymic emigrant (naïve)-type cell numbers might suggest that the developmental defects of the thymus in A-T patients should be related to this T and B cell status.