Research Article

Possible Role of Regulatory T Cells ($CD4^+$ and $CD25^+$) in Pathogenesis of Childhood Immune Mediated Thrombocytopenia

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Abstract
Immune mediated thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized by production of auto-antibodies against platelet antigens. It is obvious that regulatory T cells (Tregs) have a major role in controlling immune homeostasis and preventing autoimmunity. To investigate the frequency of Tregs, twenty newly diagnosed, twenty chronic ITP children and twenty age and sex matched healthy controls were recruited. The peripheral blood mononuclear cells were isolated and the proportion of Tregs was defined by flow cytometry method. Results showed that the frequency of Tregs significantly decreased in newly diagnosed and chronic ITP patients compared to those in healthy controls. It could be concluded that Tregs might play a role in the pathogenesis of ITP.
Keywords: Immune mediated thrombocytopenia**,** autoimmune bleeding

Introduction
Childhood immune thrombocytopenic purpura (ITP) is one of the most common benign hematologic disorders, which is characterized by the isolated, immune-mediated thrombocytopenia and mucocutaneous bleeding [1].

Acute ITP in children is typically a self-limited disorder that resolves within several weeks to months. However, approximately 20%–30% of children have persistent thrombocytopenic states for more than 6–12 months, which is called the chronic ITP [2].
To maintain the immune tolerance and to prevent autoimmune disease, $CD4^+$ and $CD25^+$ regulatory T cells (Treg), play a fundamental role[3].

Decreased number of Tregs and impairment of Tregs function have been reported in patients with various autoimmune diseases, such as systemic lupus erythematosus, rheumatic arthritis, multiple sclerosis, and diabetes as well as immune thrombocytopenic purpura[4].

Subjects and methods
Subjects
Twenty newly diagnosed ITP children (12 males and 8 females, with mean age of 6.3±2.9 years), and twenty chronic ITP children (10 males and 10 females, with mean age of 7.8±3.1 years) who referred to pediatric department and outpatient clinic Minia University hospital were enrolled.

The ITP was diagnosed based on the clinical evaluation, platelet count $<100\times10^9$/L in CBC, normal bone marrow megakaryocytes without any morphological evidence of dysplasia and no secondary disease that could be associated with thrombocytopenia. ITP patients were classified into newly diagnosed and chronic according to disease duration. Newly diagnosed patients with disease duration 0-3 months and chronic patients with disease duration more than 12 months.

Twenty age and sex matched healthy volunteers were concluded as control group. Informed consent was obtained from the parents of each participant prior to the study.

Isolation and staining of Peripheral Blood Mononuclear Cells

The blood samples were collected in Ethylene diamine tetra acetic acid (EDTA) containing tubes. Peripheral blood mononuclear cells (PBMCs) were isolated and were washed once with phosphate buffered saline and prepared for surface staining.

Cells were stained with combinations of the following monoclonal antibodies: anti-CD4 fluorescein isothiocynate (FITC), anti-CD25 Phycoerythrin (PE) (Boster Biological Technology, Ltd. 40459 Encyclopedia Circle Fremont, CA 94538 USA). Cells were acquired in the flow cytometer BD FACSCanto™ Ⅱ (Becton, Dickinson and Company, BD Biosciences, San Jose, CA 95131 USA).

Statistical analysis

Comparison between patients and healthy controls was carried out with student pair t-test and for more than two groups, ANOVA test was used. For correlation analysis, Pearson correlation test was performed. P-values less than 0.05 were considered significant. Statistical analysis was performed using SPSS program (statistical package for social sciences) software version 22.

Results

The results showed that mean platelet volume (MPV) significantly increased in newly diagnosed (11.9±1.2) $p< 0.05$ and chronic (11.1±1.7) $p< 0.05$ groups when compared to control group (9.6±1.2). There was significant increase in platelet distribution width (PDW) in newly diagnosed ITP (15.5±1.7) $p< 0.05$ when compared to control group (14.4±1.7), and there was significant decrease in plateletcrit (PCT) in newly diagnosed (0.03±0.01) $p< 0.05$ and chronic (0.07±0.03) $p< 0.05$ ITP groups when compared to control group (0.2±0.8).

The frequency of $CD4^+CD25^+$ Tregs was determined in both patient and control groups. The results showed that the frequency of Tregs significantly decreased in newly diagnosed (2.3±0.9) $p< 0.05$ and chronic ITP patients (2.5±1) $p< 0.05$ compared to those in healthy controls (5.4±1.2).

Our results also showed statistically significant correlations between Tregs percentage and PDW percentage ($r= -0.568$, $p= 0.027$) in newly diagnosed ITP group.

Discussion

Although the role of regulatory T cells in immune tolerance in healthy individuals appears to be well established, which would lead to the hypothesis that failure of the regulatory T cell system might induce autoimmunity[4].

The present study showed significant increase in MPV in newly diagnosed and chronic ITP groups when compared to control group, significant increase in PDW in newly diagnosed ITP group when compared to control and significant decrease in PCT in newly diagnosed and chronic groups compared to control. These results were similar to Mingfeng et al., (2013) who investigated the detection value of platelet parameters including platelet counts, PDW, MPV, P-large cell ratio and PCT in thrombocytopenia disease[5].

There was significant decrease in Tregs in newly diagnosed and chronic groups of ITP when compared to control group. These results were in agreement with Fahim and Monir (2006), Chang et al., (2010) and Wang et al., (2011). They suggested that Tregs might play a role in the pathogenesis of ITP [6,7,8].

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