Role of Circulating CD4$^+$ CD25$^{\text{high}}$ Foxp3$^+$ Regulatory T-Cells in Paediatric Asthma

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Abstract

**Background:** The role of T-Helper 2 (Th2) cells in the pathogenesis of allergy and asthma has been well described. However, the immunologic mechanisms that down modulate and protect against the development of these disorders are poorly characterized. A spectrum of CD4$^+$ T cells, including FOXP3-positive CD4$^+$CD25$^{+}$ T regulatory cells (Tregs) might play a critical role in regulating these diseases.

**Objective:** To investigate the role of CD4$^+$CD25$^{\text{high}}$ FoxP3 Tregs in the pathogenesis of pediatric asthma.

**Methods:** The study included 24 asthmatic children, 12 had mild intermittent asthma and 12 were of severe persistent asthma. In addition, 12 healthy subjects were used as controls. All patients were subjected to clinical examination and laboratory investigations including complete blood count with differential leucocytic and absolute eosinophilic count, serum total IgE level by ELIZA and flow cytometry was used to study the frequency of Tregs in peripheral blood lymphocytes of all studied groups using specific markers: cell-surface CD25 and CD4 expression and cytoplasmic FoxP3 expression.

**Results:** It was noticed a significant decrease in CD4$^+$CD25$^{+}$ % and CD4$^+$CD25$^{\text{high}}$ % in both mild intermittent cases and severe persistent asthmatic patients when compared to healthy controls. FoxP3 expression in Tregs was significantly lower in CD4$^+$CD25$^{\text{high}}$ T-cells of mild asthmatic patients when compared to control group. While the FoxP3 expression in Tregs was non-significantly lower in CD4$^+$CD25$^{\text{high}}$ T-cells of severe asthmatic patients. Tregs cells % was correlated significantly with mild asthma. While it did not show correlation with severe asthma. An inverse correlation between FoxP3 protein expression was revealed within CD4$^+$CD25$^{\text{high}}$ T-cells and total serum IgE when analyzed for all subjects. However, when correlation analysis was performed in each studied group separately, no significant correlation was found between FoxP3 expression and total serum IgE levels and there was no correlation between FoxP3 protein expressions within CD4$^+$CD25$^{\text{high}}$ T-cells and eosinophilic count was noticed.

**Conclusion:** The correlation of CD4$^+$CD25$^{\text{high}}$ FoxP3 Tregs with asthma pathogenesis indicates that it is important to evaluate Tregs in allergic asthmatic children. Greater understanding of the molecular and immunological mechanisms underlying the CD4$^+$CD25$^{\text{high}}$ FoxP3 Tregs might contribute the development of treatment modalities to influence disease processes of bronchial asthma in children as a future therapeutic target.

**Key words:** Asthma, children, CD4+CD25$^{\text{high}}$ regulatory T cells, FoxP3, flow cytometry.
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Introduction

Asthma is a chronic inflammatory disease of the airways characterized by bronchial hyperresponsiveness to a number of non-specific stimuli. This leads to recurrent episodes of wheezing, shortness of breath, chest tightness, and cough. Worldwide, more than 300 million individuals are suffering from the disease and its prevalence is increasing, especially among children (Provoost et al., 2009). Many cells and cellular elements play a role in particular, mast cells, eosinophils, T-lymphocytes, macrophages, neutrophils, and epithelial cells (La Rosa and Orange, 2007).

New insights in the pathogenesis of asthma suggest the role of lymphocytes. Airway inflammation in asthma may represent a loss of normal balance between two opposing populations of T-helper lymphocytes; Th1 and Th2. Th1 cells produce interleukin (IL-2) and interferon alpha (IFN-α), which are critical in cellular defense mechanisms in response to infection. Th2 generates a family of cytokines that can mediate allergic inflammation (La Rosa and Orange, 2007).

Allergic diseases are caused by the over development of Th2 biased immune responses in susceptible individuals. A number of recent studies indicate that regulatory T-cells play an important role in controlling such Th2 biased responses not only in animal models, but in humans as well (Milena et al., 2011).

Regulatory T-cells (Tregs) are a subgroup of T-lymphocytes with immune suppressive properties on effector T-cells (O’Garr and Vieira, 2004). They are found within the CD4+CD25high population, whereas CD4+CD25low cells constitute a heterogenous population of activated T-cells (Baecher – Allan et al., 2001). A further hallmark of CD4+CD25high Tregs is the selective expression of the forkhead/winged helix transcription factor (FoxP3) (Paust and Cantor, 2005). It serves as a master and critical regulator for Treg development and function and is currently found to be the most specific Treg marker (Hori et al., 2003).

It has been shown, in both animals and humans that the absence of FoxP3 is associated with the development of immune abnormalities, such as severe allergic inflammation and high immunoglobulin E (IgE) levels (Chatila, 2005).

By measuring mRNA levels, few studies examined the FoxP3 expression in the pathogenesis of asthma (Lee et al., 2007). Although this real-time PCR analysis can be informative, it was limited to a quantitative measure of mRNA levels per cell population. Recently, it became possible to measure with great accuracy the intracellular FoxP3 protein levels by using an anti-FoxP3 antibody. So far, it has not been investigated whether FoxP3 protein levels are related to human allergic lower airway diseases or not (Provoost et al., 2009).

CD4+CD25high lymphocytes comprise naturally occurring and adaptive Tregs (Blueston and Abbas, 2003). Naturally Tregs develop in the thymus, whereas adaptive tregs are suggested to be generated in the periphery form naive T-cells after antigen encounter (Baecher – Allan et al., 2004) or by rapid turnover of memory CD4+ T-cell populations (Vukmanovic – Stejic et al., 2006). Naturally Tregs are suggested to control immune responses in homeostatic conditions, whereas adaptive Tregs are believed to suppress Th1/Th2 effector cells at sites of inflammation through IL-10, transforming growth factor beta (TGF-β), or both (Van Oosterhout and Bloksma, 2005).

Several studies show that there is functional impairment of Tregs in asthmatic patients (Hartl et al., 2007). Treg-cell impairment is associated with increased DNA methylation of
Forkhead box transcription factor 3 (Foxp3), a key transcription factor in Treg-cell activity (Nadeau et al., 2010)

There is considerable interest in the therapeutic application of Tregs to allergic or asthmatic patients with the aim of providing a specific, more effective, safer and long lasting treatment to control disease symptoms (Xystrakis et al., 2007).

The current study was designed to investigate the role of CD4\(^+\)CD25\(^{high}\) FoxP3 Tregs in the pathogenesis of pediatric asthma using flowcytometric analysis and to evaluate the level of eosinophilic count and serum total IgE in relation to degree of asthma.

**Patients and Methods**

The present study included 24 asthmatic children; 14 males and 10 females ranged from 3-8 years with mean age (5.5±2.5). They were selected from the outpatient clinic and inpatient of pediatric department, Al Zahraa University hospital in the period from 2009 to 2010. They were classified into two groups based on Guidelines for Diagnosis and Management of Asthma (Expert Panel Report II, 1997) where group I included 12 patients with mild intermittent asthma treated with short acting beta agonist while group II were 12 patients with severe persistent asthma treated with inhaled corticosteroid. The patients were compared with age matched 12 clinically healthy normal control children exclusion criteria included patients having any clinical features suggesting another pulmonary process. Written consents were signed by parents of the participant children after explaining the nature of the study to them. All children enrolled in the present study were subjected to full medical history and thorough clinical examination.

**Laboratory investigations:** 4 ml of venous blood were aspirated from each patient and control, 2 ml of blood were added to EDTA solution for estimation of complete blood picture and CD4\(^+\) CD25\(^{high}\) FoxP3\(^+\) Tregs. Remaining blood samples were centrifuged for 10 min at 1500 rpm then serum was separated and the aliquots were stored at -20°C until total IgE assayed.

The following investigations were performed to all studied groups:
2. Serum total IgE level by Enzyme Immune Assay technique by SLC Spectra using BioCheck Kits[ BC-1035 from USA , ] ( normal rang 0-70 IU/ml).
3. Frequency of CD4\(^+\) CD25\(^{high}\) Tregs and FoxP3 expression by flowcytometric analysis (Data acquisition and analysis were performed on EP-ICS XL-USA flow cytometry using SYSTEM II version 3 software).

**Flow cytometric analysis of Regulatory T cells**

Fresh EDTA sample was used for evaluation of Treg cells: colour analysis of the peripheral lymphocytes was performed using flowcytometry (Coulter-Epics XL) with appropriate monoclonal antibodies (antiCD25-Phycoethrin),(anti CD4-Fluroescin isothiocyanate) obtained from Becton Dickinson Corporation.

FoxP3-PE-Cy5 (eBioscience, San Diego, CA, USA) was used for intracellular FoxP3 expression.In brief 50 \(\mu\)l of whole anticoagulated blood was lysed using 1 mL IQ test lysing reagent (Beckman Coulter, USA) followed by washing with phosphate buffer saline (PBS) (Oxoid, England). After that, the cells were stained with combinations of the following antibodies (5\(\mu\)l each): anti-CD25-PE, anti-CD4-FITC, anti-CD8–PE-Cy5 and FITC, PE and PE-Cy5 isotype controls .The test tubes were then incubated in the dark for 20 minutes followed b washing with PBS. For intracellular staining of FoxP3-PE-Cy5: anticoagulated whole blood were fixed and permeabilized with the use of FoxP3 Staining buffer Set (eBioscience,
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San Diego, CA, USA) according to the manufacturer’s instructions with certain modifications. In brief: After washing, the cell pellet was resuspended in 0.5 ml of freshly prepared fixation/permealization working solution and incubated for 30 minutes at 4°C in the dark. After that, washing once with PBS followed by washing once again with 1ml of 1X permealization buffer was done. 10 µl of FoxP3 or isotype control were added and incubated for 30 minutes at 4°C in the dark. Lastly washing with PBS followed by resuspension in PBS for analysis. Lymphocytes were gated via their forward and side scatter properties. T cells in peripheral blood were identified as described by Hartle et al. (2007). CD25^{high} expressing T cells were defined as CD4^{+} T cells that had a fluorescence intensity for CD25 of greater than 23102 and appeared as a tail to the right from the major population containing both CD4^{+}CD25^{low} and CD4^{+}CD25^{high} cells. CD4^{+}CD25^{high} T cells were referred to as Tregs, whereas CD25^{low} expressing T cells were assumed to be activated T cells. (Fig.1)

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**Figure (1):** Representative dot plots of flow cytometry and the gating strategy used (A to D). CD4^{+} cells were acquired after gating the lymphocyte population by forward- and side-scattered properties. Gating approach for discrimination of CD25^{high}, CD25^{low}, and CD25^{+} cells. The gates for the CD25^{high} and CD25^{low} populations were set by comparing the CD25 expression levels of CD4^{+} and CD8^{+} cells.
Statistical Analysis:

The statistical analysis of data was done by using statistical package for social science (SPSS) version 16 on windows XP. The description of data was done as frequency and proportion for qualitative data mean ± SD for quantitative data. The analysis of data was done to test statistical significant difference for quantitative data using student’s t test. For qualitative data [frequency & proportion] chi-square test was used. Measuring the mutual correspondence between two values was done using the Pearson’s correlation coefficient. P value was considered significant if ≤ 0.05 at confidence interval of 95% (Sokal and James, 1995).

Results:

The results of the present study are shown in the tables from 1 to 3 and figures from 2 to 4.

The two groups of asthmatic patients (mild intermittent and severe persistent) had a significantly higher peripheral eosinophilic count (P <0.05) compared with the healthy control group (462±51, 511±81 and 135±15 cells/mm3) respectively. On the other hand, there was no significant difference (P> 0.05) on comparing mild asthmatic with severe asthmatic children regarding eosinophilic count (462 ± 51 versus 511 ± 81 cells/mm3). (Table 1, Fig.2).

Concerning total serum IgE levels, they were significantly higher (P < 0.05) in the children with mild intermittent and severe persistent asthma compared with control group (476±213, 760±290 and 31±11 IU/ml). Also there was a significant difference (P < 0.05) on comparing mild asthmatic cases with severe asthmatic children for total IgE (476 ± 213 versus 750 ± 290 IU/ML). (Table 1, Fig.2)

It was noticed that, there was a significant decrease in CD4⁺CD25⁺ % and CD4⁺CD25 high % in both mild cases (6.11 ±1.83 % and 1.44± 0.64% ) and severe asthmatic Patients (8.98 ±1.21 % and 3.12 ±0.92 %) when compared to healthy controls (11.66 ±2.53 % and 5.53 ±1.65 %) ; ( P <0.001, P < 0.05) respectively. (Table 2, Fig.3)

FoxP3 expression in Tregs was significantly lower(P< 0.001) in CD4⁺CD25high T-cells of mild asthmatic patients (30±11%) when compared to control group (60± 8%). While the FoxP3 expression in Tregs was non- significantly lower in CD4⁺CD25high T-cells of severe asthmatic patients (48 ±7.88%). Moreover it was revealed that FoxP3 expression in Tregs in mild asthmatic patients was almost half that of healthy subjects. (Table 2, Fig. 4).

CD4⁺CD25low FoxP3- responsive or effectors Tcells (Teffs) were significantly increased (P <0.05) in both mild and severe asthmatic groups (12 ± 4.22 %, 16±3.36%) when compared to the controls (8±1.4%). (Table 2, Fig.3).

Considering the data from all the studied patient groups (mild and severe asthmatic) it was found that disease severity was correlated with peripheral eosinophilic count (r= 0.55; P <0.05 & r= 0.58; P <0.05) for mild and severe respectively. Also, it was correlated with serum total IgE (r= 0.65; P <0.001 & r= 0.75; P <0.001) for mild and severe respectively. Thus, higher eosinophilic count and total IgE level were correlated with poorer asthma control, with a higher correlation for total IgE than eosinophilic count. (Table 3)

Regarding the relationship between the percentage of FoxP3-positive cells and disease severity, it was clear that Tregs cells % was correlated significantly with mild asthma (r = 0.71, P < .001) while it did not show correlation with severe asthma (r = 0.22, P = 0.37). (Table 3)

An inverse correlation was revealed between FoxP3 protein expression within CD4⁺CD25high T-cells and total serum IgE (r = - 0.53, P < 0.05) when analyzed for all subjects. However, when correlation analysis was performed in each studied
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group separately, no significant correlation was found between FoxP3 expression and total serum IgE levels. On the other hand, there was no correlation between FoxP3 protein expressions within CD4^+CD25^{high} T-cells and eosinophilic count was noticed.

**Table (1): Comparison between asthmatic groups and control.**

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th>Mild intermittent asthmatics</th>
<th>Severe persistent asthmatics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients Number</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Male/Female(no)</td>
<td>7/5</td>
<td>8/4</td>
<td>9/3</td>
</tr>
<tr>
<td>Peripheral blood Eosinophilic count (cells/mm³)</td>
<td>135(±15)</td>
<td>462(±51)^*</td>
<td>511(±81)^*</td>
</tr>
<tr>
<td>Total IgE (IU/ML)</td>
<td>31(±11)</td>
<td>476(±213)^*</td>
<td>760(±290)^*F</td>
</tr>
</tbody>
</table>

^*P<0.05 compared with control group. ^TP < 0.05 compared with mild intermittent asthma.

![Figure (2): Peripheral blood eosinophilic count and total serum IgE levels in the studied groups.](image)

**Table (2): Comparison of Tregs T-cells in the patients and control group**

<table>
<thead>
<tr>
<th>Studied variables</th>
<th>Healthy controls (N=12)</th>
<th>Mild asthmatics (N=12)</th>
<th>Severe asthmatics (N=12)</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4^+CD25^+</td>
<td>11.66 ±2.53 %</td>
<td>6.11 ±1.83 %</td>
<td>8.98 ±1.21 %</td>
<td>P1&lt; 0.001 P2&lt; 0.05 P3&lt; 0.05</td>
</tr>
<tr>
<td>CD4^+CD25^{high}</td>
<td>5.53 ±1.65 %</td>
<td>1.44 ±.64%</td>
<td>3.12 ±0.92 %</td>
<td>P1&lt; 0.001 P2&lt; 0.05 P3&lt; 0.05</td>
</tr>
<tr>
<td>CD4^+CD25^{low}FoxP3^+</td>
<td>8 ± 1.4 %</td>
<td>12 ± 4.22 %</td>
<td>16 ± 3.36%</td>
<td>P1&lt; 0.05 P2&lt; 0.05 P3&lt; 0.05</td>
</tr>
<tr>
<td>CD4^+CD25^{high}FoxP3^+</td>
<td>60 ± 8%</td>
<td>30±11%</td>
<td>48 ±7.88%</td>
<td>P1&lt; 0.001 P2&gt; 0.05 P3&lt; 0.05</td>
</tr>
</tbody>
</table>

P1= between controls and mild intermittent asthma, P2=between controls and severe persistent asthma and, P3= between mild and severe asthmatic patients.
Figure (3): Flow cytometric analysis of Treg% (F gate in region 2) and Teff% (G gate in region 2) in circulating CD4\(^+\) T cells. Representative data of a control child (A), mild intermittent asthma (B) and severe persistent asthma (C).

Figure (4): Flowcytometric analysis of FOXP3 expression in circulating CD4\(^+\)T cells. Comparison of percentages of FOXP3-positive CD25\(^{high}\) T cells in a CD4\(^+\) T-cell population between (A) control subjects (n=12), (B) patients with mild intermittent asthma (n =12) and (C) patients with severe persistent asthma (n=12). Thick horizontal bars indicate the mean for each group.
Table (3): Correlation between severity of asthma and immune parameters in the patient groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mild intermittent asthma (N=12)</th>
<th>Severe persistent asthma (N=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Peripheral eosinophilic count</td>
<td>0.55</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Serum total IgE</td>
<td>0.65</td>
<td>&gt; 0.001</td>
</tr>
<tr>
<td>FoxP3 protein expression</td>
<td>0.71</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Discussion

Airway inflammation in asthma is characterized by activation of Th2 cells, IgE production and eosinophilia (Robinson, 2009). T-regulatory cells (Tregs) are known to play a key role in balancing immune responses to maintain peripheral tolerance against harmless antigens or allergens (Bacchetta et al., 2007). Peripheral blood CD4⁺CD25⁺ Tregs were found to suppress Th2 cytokine production from both atopic and non atopic individuals (Bellinghausen et al., 2003).

FoxP3 serves as a master regulator for Treg development and functions and is currently found to be the most specific Treg marker (Hori et al., 2003).

In the present study, we aimed to investigate and evaluate the role of CD4⁺CD25⁺ high FoxP3⁺ Tregs in the pathogenesis of pediatric asthma using flowcytometric analysis as a rapid, easy, inexpensive and accurate technique. From the current study, it was noticed that there was a variable significant decrease in CD4⁺CD25⁺ % and CD4⁺CD25⁺ high % in both mild intermittent and severe persistent group when compared to healthy control; P < 0.001 and P < 0.05 respectively.

These findings were consistent with Lee et al. (2007), who pointed out that children with allergic disease had fewer Tregs cells than control subjects.

In a study done by Hartl et al. (2007) to investigate the role of Treg cells in childhood asthma, the levels of CD4⁺CD25⁺ Treg cells in peripheral blood and bronchoalveolar lavage fluid (BALF) were measured where CD4⁺CD25⁺ Treg cells were decreased in the BALF of children with asthma vs. those with cough or no lung disease. Furthermore, the CD4⁺CD25⁺ Treg cells in children without asthma suppressed proliferation and cytokine production by pulmonary Th2 cells, but the Treg cells from asthmatic children did not suppress these T cells. Inhaled corticosteroids restored this suppressive capacity. This is strong evidence that CD4⁺CD25⁺ Treg cell function is locally impaired in asthmatic children (Lee et al., 2007).

These findings of the current work could fit with the hypothesis that in asthma, which is characterized by an unwanted inflammatory reaction there is an underlying relative deficiency in Tregs allowing high numbers of Th2 cells to develop (Barnes, 2008).

On the contrary Provoost et al. (2009) reported no difference in the number of circulating CD4⁺CD25⁺ or CD4⁺CD25⁺ high T-cells in control subjects vs asthmatic patients where it is unclear if factors such as race, age, disease severity, or treatment contribute to these differences. In addition, and probably of more importance, the peripheral blood CD4⁺CD25⁺ T-cells represent a heterogeneous population and contain Treg, as well as activated effector T-cells.

The present work demonstrated that FoxP3 expression was significantly lower in CD4⁺CD25⁺ high Tregs in group I (30±11%) when compared to control
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Total Serum IgE was the original screening test for allergy, but has been superseded by newer more specific tests. However a total IgE level exceeding 70 IU/ml is highly suggestive of allergy in children. Total IgE allergy testing has good predictive values in children less than 3 years of age and may be used as a screening test in this group (Yousri et al., 2011).

IN this study, similarly to other reports, we also showed that the severity of asthma correlated with total serum IgE levels. Moreover an inverse correlation between FoxP3 protein expression was revealed within CD4^{+}CD25^{high} T-cells and total serum IgE (r = -0.53, P < 0.05) when analyzed for all subjects. However, when correlation analysis was performed in each patient group separately, no significant correlation was found between FoxP3 expression and total serum IgE levels. On the other hand there was no correlation between FoxP3 protein expressions within CD4^{+}CD25^{high} T-cells and eosinophilic counts were noticed.

On the contrary, Provoost et al. (2009) found no significant correlation between FoxP3 expressions and total serum IgE levels. Also Hartl et al. (2007) did not find associations between total or specific IgE levels and percentages of CD4^{+}CD25^{high} T cells in BALF or peripheral blood. Lee et al. (2007) and Ito et al. (2009) recorded a positive correlation between numbers of CD4^{+}CD25^{+}T cells and serum IgE levels where they attributed that difference in observed frequencies with other studies might be related to the disease status.

T-cells are critical for the initiation and maintenance of the mature asthmatic inflammatory response. Complex interactions between T and B lymphocytes and antigen presenting cells lead to inflammation, cytokine production, IgE production and bronchial hyperresponsiveness (Donovan and Finn, 1999).

It has been demonstrated that both mouse and human naive CD4^{+}CD25^{−} T cells can be ‘converted’ to CD4^{+}CD25^{+}FoxP3^{+} Treg cells. Adoptive transfer of these Treg cells, which have been polarized from their own CD4^{+}CD25^{−}T cells, back to patients with allergic disease, could be an ideal therapeutic approach (Alison et al., 2010).

Conclusion

The correlation of CD4^{+}CD25^{high} FoxP3 Tregs with asthma pathogenesis indicates that it is important to evaluate Tregs in allergic asthmatic children. Corticosteroid treatment in asthmatics might increase the FoxP3 expression within CD4^{+}CD25^{high} Tregs cells. Additionally, the use of inhaled corticosteroids allowed for the
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CD4⁺CD25⁺ T- regulatory cells to become functional, indicating a therapeutic mechanism for further research. Greater understanding of the molecular and immunological mechanisms underlying the CD4⁺CD25<sup>high</sup> FoxP3 Tregs might contribute the development of treatment modalities to influence disease processes of bronchial asthma in children as a future therapeutic target.

References


دور الخلايا الليمفاوية – ت الضابطة CD4⁺CD25⁷⁰³⁰ FoxP3 في حالات الأطفال المصابين بالربو

الخليفة: دور الخلايا الليمفاوية – ت الضابطة (CD4⁺CD25⁷⁰³⁰ FoxP3) في حالات الأطفال المصابين بالإضافي.

الهدف: تقييم مستوى الخلايا الضابطة CD4⁺CD25⁷⁰³⁰ FoxP3 في حالات الربو والأطفال.

الطرق: استخدم التدفق الخموي لدراسة نسبة الخلايا الضابطة المتقطعة CD4⁺CD25⁷⁰³⁰ FoxP3 في الحالات. كما وُضع الطلب بالخلايا المتقطعة في حالات الربو البسيط المتقطع في نفس المجموعة. كما تم قياس الأجسام المضادة IgE في جميع الحالات.

النتائج: كان هناك انخفاضًا في حالة الربو المتقوية (6.11 ± 1083%) ، و (1.44 ± 5.64) حالات الربوب شديد المتقطع (8098 ± 1021%) ، و (3.12 ± 5.92%) مع انخفاضات دال على مجموعة الأطفال الأصحاء (11.66 ± 1012%) و (5053 ± 1065) مع انخفاضات دال على الحالات. كما وجد أن هناك ارتباط عكسي بين العلاج الخامل Foxp3 والخلايا المضادة IgE في جميع الحالات. وعند الخلايا بالدم، ورد انخفاض خلق الخلايا الليمفاوية مع إرتفاع CD4⁺CD25⁷⁰³⁰ FoxP3 وعمل دراسات أخرى.

الاستنتاجات: يشير انخفاض خلق الخلايا الليمفاوية مع إرتفاع CD4⁺CD25⁷⁰³⁰ FoxP3 إلى وجود دور للمناعة في مرض الربو، وعدم قدرة الخلايا الضابطة على ضبط نشاط المرض إما بسبب نقص نسبتها أو لوجود خلل وظيفي مما يحفز لعمل دراسات أخرى.