Detection of plasmacytoid dendritic cell (pDC) content in peripheral blood and renal tissue of children with henoch–schonlein purpura and its clinical value

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ABSTRACT

Objective: To study the plasmacytoid dendritic cell (pDC) content in peripheral blood and renal tissue of children with henoch-schonlein purpura and its clinical value. Methods: 30 cases of henoch-schonlein purpura children with renal damage were enrolled in HSPN group, 30 cases of henoch-schonlein purpura children without renal damage were enrolled in NHSPN group, and 30 cases of healthy volunteers were enrolled in the control group. Then contents of pDC, Th2 cell, IL-4, IL-5, IL-10 and IL-13 in peripheral blood as well as contents of pDC, Th17 cell, IL-17, IL-21 and IL-23 in renal tissue of three groups were detected. Results: (1) pDC contents in peripheral blood of HSPN group and NHSPN group were lower than those of control group and the decrease of pDC contents in peripheral blood of HSPN group was more obvious; CD304 contents in renal tissue of HSPN group and NHSPN group were lower than those of control group and the increase of CD304 contents in renal tissue of HSPN group was more obvious; (2) Th2 cell as well as IL-4, IL-5, IL-10 and IL-13 contents in peripheral blood of HSPN group and NHSPN group were higher than those of control group and the increase of related indexes in peripheral blood of HSPN group was more obvious; Th17 cell as well as IL-17, IL-21 and IL-23 contents in kidney tissue of HSPN group were higher than those of NHSPN group; (3) in peripheral blood, pDC content was negatively correlated with Th2 cell level as well as IL-4, IL-5, IL-10 and IL-13 contents, and in renal tissue, pDC content was positively correlated with Th17 cell level as well as IL-17, IL-21 and IL-23 contents. Conclusions: Abnormal pDC content correlates with the pathogenesis of henoch-schonlein purpura, pDC content decreases in peripheral blood and will result in enhancement of Th2 cell function, and pDC content increases in kidney and will result in enhancement of Th17 cell function.

1. Introduction

Henoch-schonlein purpura (HSP) is a common autoimmune disease and its characteristic pathological change is necrotizing small-vessel vasculitis. Henoch-schonlein purpura nephritis (HSPN) is the most common complication of HSP and will cause extremely unfavorable impact on patients’ prognosis[1]. At present, the mechanisms of immunologic dysfunction in development process of HSP and HSPN have not been fully elucidated. Dendritic cell (DC) is the most powerful antigen presenting cell that can be involved in antigen uptake and processing, activate naive T cells, stimulate antigen-specific immune response and maintain immune tolerance[2]. Abnormal DC function will affect the function of Th1, Th2, Th17 and other immune cells, and thereby result in the occurrence of autoimmune diseases[3]. In the following research, the plasmacytoid dendritic cell (pDC) content in peripheral blood and renal tissue of children with henoch-schonlein purpura and its clinical value were analyzed.
2. Research materials and subjects

2.1. Research materials and instruments

Lymphocyte separation medium was from Amersham Company of the US, fluorescence-labeled monoclonal antibodies were from Abcam Company of the US, and ELISA kits were from Roche Company of the US; flow cytometer was from Becton Company of the US, PCR amplification instrument was from ABI Company of the US and ELIASA was from Bio-tek Company of the US.

2.2. Grouping and information of research subjects

Enrolling time range of the research subjects was from May 2013 to December 2014. 30 cases of henoch-schonlein purpura children with renal damage were enrolled in HSPN group and 30 cases of henoch-schonlein purpura children without renal damage were enrolled in NHSPN group. Both groups met the diagnostic criteria of henoch-schonlein purpura established by the Subspecialty Group of Nephrology, the Society of Pediatrics of Chinese Medical Association in the conference in Zhuhai in the year 2000, and whether they were complicated with renal injury or not was confirmed by pathologic biopsy. 30 cases of healthy volunteers were enrolled in the control group.

2.3. Research methods

2.3.1. Flow cytometry

Fasting peripheral blood was collected in the morning, added to lymphocyte separation medium after heparin anticoagulation and centrifuged for 20 min with centrifugal force of 500 g, mononuclear cells were drawn and separately put in flow cytometer reaction tubes, different fluorescence-labeled monoclonal antibodies were incubated respectively away from light for 15 min; after incubation, PBS wash and centrifuge were carried out twice, finally PBS containing 5% FBS was added, and percentages of pDC, Th2 and Th17 cells were detected in flow cytometer.

2.3.2. Fluorescent quantitative PCR detection

Renal biopsy tissue was taken and added to RNAiso liquid produced by Takara Company, and total RNA in the tissue was extracted according to the procedures; DEPC water was added to dissolve RNA and then reverse transcription into cDNA; cDNA specimens were diluted with deionized water in the ratio of 1:5 and then used for PCR reaction to amplify target gene CD304 and reference gene β-actin respectively. β-actin was taken as internal reference to semi-quantitatively analyze CD304 contents of HSPN group and NHSPN group. Primer sequences and annealing temperatures were shown in Table 1.

2.3.3. ELISA detection

Fasting peripheral heparin anticoagulant blood was collected in the morning, kit instructions were followed to configure concentration gradient standard substance and prepare the specimens to be detected, kit instructions were followed for sample application, incubation and plate washing, finally absorbance (OD values) at 450 nm was read in ELIASA and input into computer to get standards curves of concentration gradient - OD values, and OD values of specimens to be detected were used in the standard curves to get cytokine contents.

2.4. Statistical processing methods of the data

Data was analyzed by SPSS21.0 software. Comparison of measurement data between two groups was by *t* test and comparison of measurement data among three groups was by variance analysis. Differences were considered to be statistically significant at a level of *P*<0.05.

3. Results

3.1. pDC contents in peripheral blood and kidney tissue

Flow cytometry was used to detect pDC contents in peripheral blood, and analysis results showed that pDC contents in peripheral blood of HSPN group and NHSPN group were lower than those of control group and pDC contents in peripheral blood of HSPN group were lower than those of NHSPN group; fluorescent quantitative PCR method was used to detect CD304 contents in kidney tissue, and analysis results showed that CD304 contents in kidney tissue of HSPN group and NHSPN group were higher than those of control group and CD304 contents in kidney tissue of HSPN group were higher than those of NHSPN group (Figure 1).

### Table 1
Primer sequences and annealing temperatures of CD304 and β-actin.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Forward primers (5’→3’)</th>
<th>Reverse primers (5’→3’)</th>
<th>Annealing temperatures (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD304</td>
<td>AGTAGCGTACGTCATAGTG</td>
<td>CGTAGCTGCTGCTAGCTG</td>
<td>60.3</td>
</tr>
<tr>
<td>β-actin</td>
<td>CTGCTGCTGCTGCTGCTG</td>
<td>CAGTGGTGTAGCATG</td>
<td>58.5</td>
</tr>
</tbody>
</table>
### 3.2. Contents of Th2 cell and related cytokines in peripheral blood

Flow cytometry was used to detect Th2 cell contents in peripheral blood, and analysis results showed that Th2 cell contents in peripheral blood of HSPN group and NHSPN group were higher than those of control group and Th2 cell contents in peripheral blood of HSPN group were higher than those of NHSPN group; ELISA was used to detect contents of related cytokines in peripheral blood, and analysis results showed that IL-4, IL-5, IL-10 and IL-13 contents in peripheral blood of HSPN group and NHSPN group were higher than those of control group and Th2 cell contents in peripheral blood of HSPN group and NHSPN group were higher than those of control group (Table 2).

### 3.3. Correlation between pDC content and contents of Th2 cell as well as related cytokines in peripheral blood

pDC content in peripheral blood was taken as independent variable and contents of Th2 cell as well as IL-4, IL-5, IL-10 and IL-13 were taken as dependent variables for linear regression analysis, and analysis results showed that pDC content was negatively correlated with Th2 cell level as well as IL-4, IL-5, IL-10 and IL-13 contents, and related coefficients were -0.823, -0.795, 0.801, -0.787 and -0.849 respectively.

### 3.4. Contents of Th17 cell and related cytokines in kidney tissue

Flow cytometry was used to detect Th17 cell contents in kidney tissue, and analysis results showed that Th17 cell contents in kidney tissue of HSPN group were higher than those of NHSPN group; ELISA was used to detect contents of related cytokines in kidney tissue, and analysis results showed that IL-17, IL-21 and IL-23 contents in kidney tissue of HSPN group were higher than those of NHSPN group (Table 3).

### 4. Discussion

Dendritic cell (DC) is the APC cell with currently the most powerful antigen presenting function, and plasmacytoid dendritic cell (pDC) is one kind of dendritic cells[4]. In recent years, more and more studies believe that dendritic cells are closely related to the occurrence of allergic diseases and are the initial factors of allergic reactions[5]. CD304 is a marker molecule on the surface of pDC and a member of Semaphorin3 type superfamily. CD304 was detected to analyze pDC contents in peripheral blood and kidney tissue of henoch-schonlein purpura children, and results indicated that pDC contents in peripheral blood and kidney tissue showed different trends, pDC contents in peripheral blood of HSPN group and NHSPN group were lower than those of control group and the decrease of pDC contents in peripheral blood of HSPN group was...
more obvious; that CD304 contents in kidney tissue of HSPN group and NHSPN group were higher than those of control group and the increase of CD304 contents in kidney tissue of HSPN group was more obvious, which indicated that in development process of HSP, pDC continued to migrate from peripheral blood to the target organ; when disease progressed into henoch-schönlein purpura nephritis, large amounts of pDC infiltrated in kidney tissue.

Based on above research results, pDC distribution is uneven in peripheral blood and kidney tissue, which will cause unbalance of immune function and the occurrence of autoimmune diseases. Existing research believes that the occurrence of henoch-schönlein purpura is related to Th2 migration[6]. The generation of antigen specific IgE depends on Th2 cells and Th2 cells can enhance humoral immune response[7]; meanwhile this type of cells can generate large amounts of IL-4, IL-5, IL-10, IL-13 and other cytokines, and thereby exert the function of signaling molecule in allergic reaction and other specific immune response[8]. In the research, analysis of contents of Th2 cell and related cytokines in peripheral blood showed that Th2 cell contents as well as IL-4, IL-5, IL-10 and IL-13 contents in peripheral blood of HSP group and NHSP group were higher than those of control group and the increase of Th2 cell contents as well as IL-4, IL-5, IL-10 and IL-13 contents in peripheral blood of HSPN group was more obvious.

Studies have shown that IL-12 synthesized and secreted by pDC can be involved in the regulation of helper T cell differentiation and maturation, manifested as promoting Th0 cell differentiation and maturation to Th1 cell and causing Th1/Th2 balance offset to Th1[9,10]. In progression process of HSP, pDC migration and infiltration to target organ will cause decreased pDC content in peripheral blood, thereby cause diminished effect of its function to enhance Th1 function and indirectly cause enhancement of Th2 function, increased secretion of a variety of cytokines, enhancement of humoral immune response and generation of large amounts of antigen-specific antibodies[11,12]. In order to preliminarily make clear whether pDC was involved in the regulation of Th2 function in peripheral blood, correlation between pDC contents and contents of Th2 cell as well as related cytokines was analyzed, and results showed that pDC content was negatively correlated with Th2 cell level as well as IL-4, IL-5, IL-10 and IL-13 contents, which indicated that pDC had negative regulating effect on Th2 cell function in peripheral blood.

Infiltration of pDC in kidney can cause tissue injury through multiple pathways[13]. Th17 is a new type of CD4+ helper T cell subgroup discovered in recent years, mainly secretes IL-17, IL-21, IL-22 and other cytokines, and can induce infiltration of inflammatory cells in mononuclear macrophages, endothelial cells and smooth muscle cells, and increase the generation of multiple inflammatory factors and chemotactic factors[14,15]. In the research, analysis of contents of Th17 and related cytokines in kidney tissue showed that Th17 cell contents as well as cytokines IL-17, IL-21 and IL-23 contents in kidney tissue of HSPN group were higher than those of NHSP group, which indicated that Th17 cells were involved in renal injury process of henoch-schönlein purpura patients. Further analysis of the correlation between pDC content and Th17 cell function in kidney tissue showed that pDC content was positively correlated with Th17 cell level as well as IL-17, IL-21 and IL-23 contents, which indicated that pDC had positive regulating effect on Th17 cell function in kidney tissue. In the development process of henoch-schönlein purpura, pDC migrated to kidney and caused renal injury through enhancement of Th17 cell function.

Based on above discussions, it is believed that abnormal pDC expression correlates with the pathogenesis of henoch-schönlein purpura, pDC content decreases in peripheral blood and will result in enhancement of Th2 cell function, and pDC content increases in kidney and will result in enhancement of Th17 cell function.

References