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Cellular immunity profile in children with congenital heart disease and bronchopneumonia: evaluation of lymphocyte subsets and regulatory T cells

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Abstract

Children with congenital heart disease (CHD) have a predisposition to suffer from respiratory tract infections, such as bronchopneumonia (BP). In this study we investigated the characterization of lymphocyte subsets and regulatory T cells (Tregs) in these children. The frequencies of lymphocyte subsets and regulatory T cells were detected in peripheral blood of 400 children patients [100 with BP only, 100 with BP and CHD (BPCHD), 100 with BP and heart failure (BPHF), 100 healthy volunteers] by using three-color flow cytometry. In BPHF and BPCHD groups, lymphocyte subsets characterization of patients was analogous, with lower levels of CD3+, CD3+CD4+, CD3+CD8+, and CD4+/CD8+ ratio but higher levels of CD19+ and CD3–CD16+CD56+ in comparison to BP patients. The differences of the frequencies of CD4+CD25+CD127– T-cells in the four groups were not statistically significant. It was concluded that the cellular immunity function of children with CHD was vulnerable to being damaged after having suffered from BP when compared with the children without CHD, which might be associated with blood circulation difficulties in the majority of children with CHD.

Key words: *cellular immunity, lymphocyte subsets, regulatory T cells (Tregs), bronchopneumonia, congenital heart disease.*

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Introduction

Human congenital heart disease (CHD) is a common congenital anomaly, and exhibits a gross structural abnormality of the heart or intrathoracic great vessels, which is potentially of functional significance. The development of heart disease is a complex biological process requiring the integration of cell commitment, morphogenesis, and excitation-contraction coupling, which can make the immunological profile of patients disorganized. Once the homeostasis of the immune system is destroyed, more infectious diseases, such as opportunistic infections will be induced [1]. T lymphocyte-mediated immune function plays an important role in immune response and immune regulatory function of the body. Many investigations indicated that abnormal frequencies of CD3+, CD3+CD4+, CD3+CD8+, and abnormal CD4+/CD8+ ratio reflected the changes of the body immune function [2, 3].

The quantity of T lymphocytes in infants with CHD was abnormal [4]. Middle-aged (< 55 years) out-patients with coronary heart disease showed that CD4+/CD8+ balance was distorted [5]. CD3+CD4+ T cells can be subdivided into helper T cell (Th) subpopulations (Th1, Th2, Th17, Tregs) because of different cytokine secretion patterns [6, 7]. Th1 cells induce effectively cellular immune responses by producing pro-inflammatory cytokines (interferon – IFN), interleukin (IL)-2, tumor necrosis factor α (TNF-α), which can activate macrophages and initiate inflammation. Th2 cells participate in allergy, help effectively B cells to develop into antibody-producing cells and also suppress Th1-dependent inflammation by secreting IL-4, IL-5, IL-10 and IL-13 cytokines [8]. Natural killer cells (NK), expressing CD16 and CD56, can regulate immunity by secreting interferon-C in addition to participating in innate immunity. Reduction in NK percentage made the secretion of interferon-C decreased, and then resulted in the imbalance of Th1/Th2 [9]. Immunity and tolerance cooperate in humans to maintain the immune system homeostasis. Regulatory T cells (Tregs) contribute to the process of immune suppression, a tolerance promoting mechanism. Tregs suppress autoimmune reac-

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tivation and excess inflammatory responses, which have important roles in instructing immune responses against cancer and various pathogen infections [10, 11]. Disruption of Tregs function will result in a plethora of autoimmune and inflammatory pathologies [12]. Therefore, the detection of Treg levels in human peripheral blood also has a very significant meaning. Spontaneous CD4+CD25+ regulatory T-cells are a subset of CD4+ T-cells. CD4+CD25+CD127– T cells best fit the definition of naturally occurring regulatory T cells in human peripheral blood [13]. The estimation of cellular immunity is significant in diagnosis and prognosis of some diseases. Clearly, recognition of cell-mediated immunodeficiency (CMID) may be crucial to the survival of a newborn, which often results in death due to an overwhelming infection during the first weeks of life [4]. Children with CHD have a predisposition to infections especially of the respiratory tract [14]. Respiratory congestion resulting from increased pulmonary blood flow may interfere with the clearance of infectious organisms. Respiratory tract infections caused by bacteria, viruses, fungi, or parasites, often induce pneumonia. Pneumonia is a major death cause in children, especially those younger than 5 years, accounting for up to 5 million deaths each year in developing countries [15]. Bronchial pneumonia resulting from lower respiratory infections affects the bronchial tubes and patches of the lungs. At times, irritation and inflammation can overspread into both lungs, which may make a person very sick or even cause death. However, a weakened immune system may be responsible for this condition.

Bronchial pneumonia is more common in infants, children, the elderly, and patients with compromised immune systems, affecting the respiratory system as well as the entire body. In the present study, we analyzed cellular immunity status of patients with bronchial pneumonia by flow cytometry, which was associated with congenital heart disease or heart failure.

Material and methods

This study was approved by the Ethics Committee of the Hospital and the procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000. All participants gave informed consent.

Patients

Three hundred children hospitalized with bronchopneumonia (BP) were enrolled in this study. These patients were classified as a bronchopneumonia group (BP group; 56 men and 44 women; mean ± SD ages 1.67 ±1.47 years), a BP associated with congenital heart disease group (BPCHD group; 59 men and 41 women; mean ± SD ages 0.66 ±0.6 years), and a bronchopneumonia associated with heart failure group (BPHF group; 49 men and 51 women; mean ± SD ages 0.66 ±0.5 years) according to the patients' condition. One hundred healthy children were selected as a control group (control group; 57 men and 43 women; mean ± SD ages 0.91 ±0.18 years) after physical examination, who had no history of BP, CHD, HF, use of immunosuppressants or severe heart, liver, kidney or allergic diseases. In the BPCHD group, the children presented only with early clinical presentation of congenital heart disease but without the symptoms of heart failure.

Antibodies

Cells were stained with surface mAbs against CD3, CD4, CD8, CD19, CD16CD56, CD25 and CD127 according to the manufacturer's protocol, which included PC5, PE, or FITC conjugated mouse antibodies. All of mAbs were purchased from Beckman Coulter Co., Ltd.

Flow cytometry

Three-color flow cytometry was used to detect the frequencies of lymphocyte subsets and Tregs. 10 µl of antibodies, including CD3-PC5, CD4-FITC and CD8-PE, were added into the tube which had 50 µl of anticoagulated whole blood in order to determine the frequency of T lymphocyte cells. 10 µl of antibodies containing CD3-FITC and CD16CD56-PE and 6 µl of CD19-PC5 were added successively to 50 µl of anticoagulated whole blood in order to detect the frequency of NK and B lymphocyte cells. 10 µl of antibodies, including CD4-PC5, CD25-FITC and CD127-PE, were added to 50 µl of anticoagulated whole blood in order to determine the frequency of CD4+CD25+ and CD4+CD25+CD127–. After shocked, the tubes were incubated at room temperature in the dark for 20 min. Erythrocyte lysis buffer (1 ml) was then added, followed by incubation at room temperature in the dark for 15 min. Then, the tubes were centrifuged (1200 rpm, 5 min), the supernatants were discarded and the cells were washed once with 2 ml of phosphate-buffered saline. Finally, the cells were resuspended in 500 µl of phosphate-buffered saline and analyzed by flow cytometry.

Statistical analyses

Data were analyzed using CXP 2.2 software on a flow cytometer (FC500, Beckman Coulter) and presented as mean ± SD. A statistically significant difference was defined as p value of less than 0.05. SPSS version 18.0 software was used for statistical analyses.

Results

The frequencies of lymphocyte subsets and regulatory T cells are shown in Figure 1. Patients with BP showed higher CD3+ levels ($p < 0.05$) and CD3+CD8+ ($p < 0.05$) but lower CD4+/CD8+ ratio ($p < 0.05$), and CD19+ ($p < 0.05$) than controls. Difference in CD3+CD4+ levels and CD3–CD16+CD56+ levels were not statistically significant. Compared with these patients only with BP,

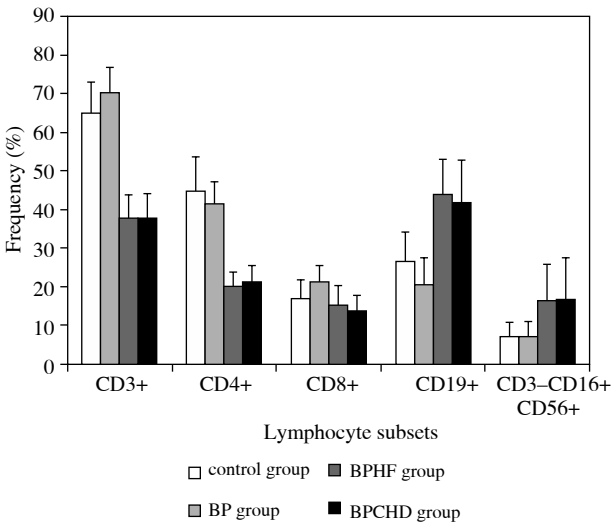

Fig. 1. The frequencies of lymphocyte subsets in each group of subjects

BP patients with complicating heart failure were characterized by lower levels of CD3+ ($p < 0.001$), CD3+CD4+ ($p < 0.001$), CD3+CD8+ ($p < 0.05$), and CD4+/CD8+ ratio ($p < 0.05$) but higher levels of CD19+ ($p < 0.001$) and CD3-CD16+CD56+ ($p < 0.01$). It is worth noting that lymphocyte subsets characterization of BP patients with CHD were analogous to that of BP patients with HF. A little difference in lymphocyte subsets characterization between BPHF patients and BPCHD patients were all not statistically significant. The BPCHD patients displayed lower levels of CD3+ ($p < 0.001$), CD3+CD4+ ($p < 0.001$), CD3+CD8+ ($p < 0.001$), and CD4+/CD8+ ratio ($p < 0.001$) but higher levels of CD19+ ($p < 0.001$) and CD3–CD16+CD56+ ($p < 0.01$) in comparison to BP patients. Data from a representative donor are presented here (Fig. 2).

In the BP patients with complicating HF, the frequencies of CD4+CD25+CD127– T-cells were higher than those of other three groups. CD4+CD25+CD127– T-cells level of the BPCHD patients were higher than that of controls and BP patients. However, all of these differences were not statistically significant (Fig. 3).

Discussion

Bronchopneumonia, a condition in which the bronchial tubes become inflamed, is a common and frequent disease during the infantile period. At times a serious condition, such as complicating heart failure, is most dangerous and life-threatening. Although anti-infection treatment has achieved considerable progress in the recent years, severe pneumonia is still one of the great challenges that the clinical therapy of respiratory system infection faces. More

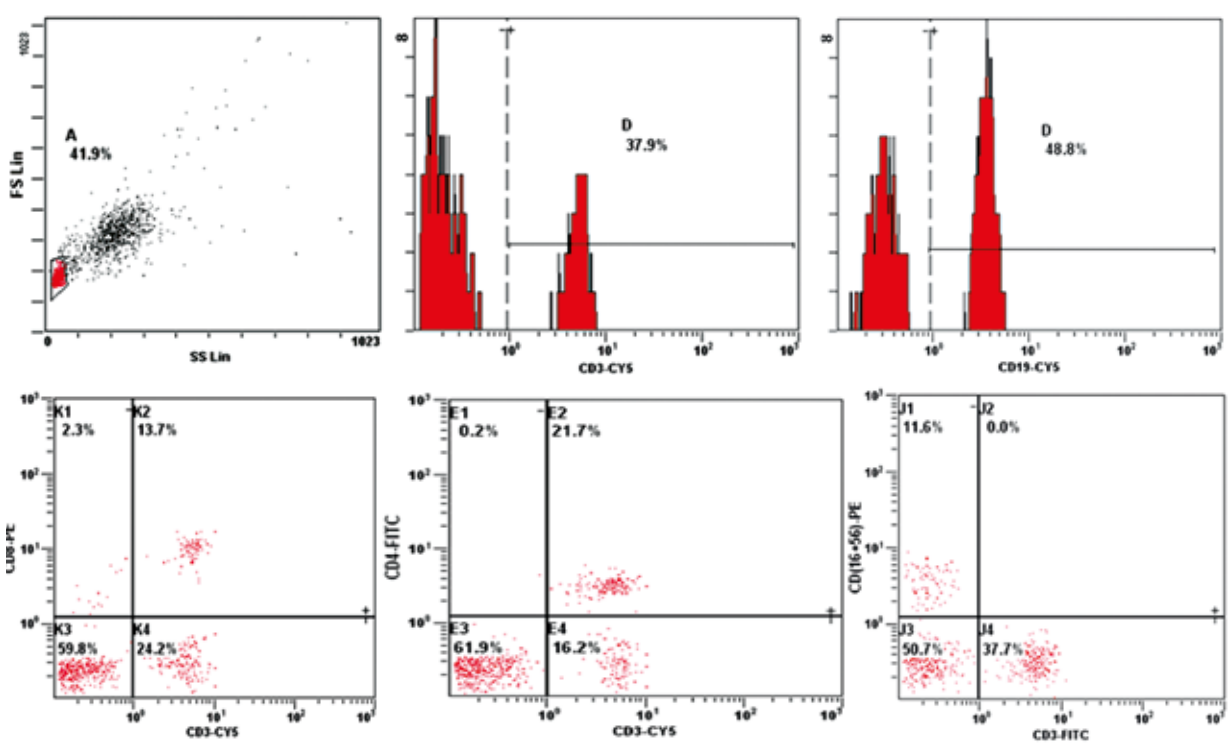

Fig. 2. The flow cytometric analysis of lymphocyte subsets in peripheral blood of a representative donor

and more studies have revealed that the pathogenesis of BP was connected with not only the infringement of pathogens but also the body immunity. Immunity balance, including cellular and humoral immunity, plays a crucial role in the occurrence and development of the disease. The hypoplasia or dysfunction of the immunity system may be responsible for bronchopneumonia in childhood.

In this study, according to whether complicating heart failure was present or not, the patients were divided into a common BP group and a severe bronchopneumonia group (BPHF group). The results showed that BP patients had abnormal distribution of lymphocyte subsets in comparison to the controls. The findings about the higher levels of CD3+CD8+ and decreased CD4+/CD8+ ratio and CD19+ levels indicated that bronchial pneumonia of these patients was mainly induced by virus infection. After infection, T lymphocyte cells proliferated and differentiated rapidly, and then the total T-cell percentage was significantly increased, while B-cell percentage was significantly decreased. However, in the BPHF group, all of patients presented with lower CD3+, CD3+CD4+, CD3+CD8+ levels but higher levels of CD19+ and CD3–CD16+CD56+ than BP patients, which demonstrated that cell-mediated immunity was distinctly suppressed and immune function was disordered as the deterioration of bronchial pneumonia. The state of the body's immune balance depends on mutual coordination and mutual restriction of the various lymphocyte subsets, which can form a moderate immune response. Abnormal frequency of lymphocyte subsets resulting from the infection of bacteria or virus may cause immune dysfunction, and furthermore, result in the development and deterioration of disease. Especially, the serious imbalance of T lymphocyte percentage is more likely to lead to extensive tissue injuries [16]. Immunosuppression makes it difficult to inhibit the development of infectious disease [17]. The occurrence and development of heart disease can usually make the immunity function of the patient disorder. There was evidence that an altered CD4+/CD8+ ratio and decreased CD3+ levels were related with coronary heart disease [5]. CD4+/CD8+ ratio was inverted in patients with myocardial infarction, which usually returned to normal within 2 days following admission to the coronary care unit [18]. It had been reported that there was an increased susceptibility to infection in children with congenital heart disease [14], and the predilection of these patients to infection might be explained partly by an underlying immune function disorder [19]. In the present study, after having suffered from BP, the patients with congenital heart disease exhibited a distinct reduction in total T lymphocyte percentage and helper T-cells when compared with that of the BP group. Although they had not presented with heart failure symptoms, lymphocyte subsets characterization of which was similar to that of those BPHF patients. In this condition, the children with CHD after having suffered from BP were more likely to develop into heart failure.

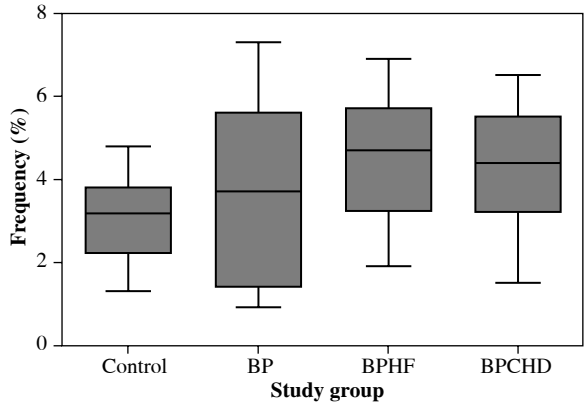

Fig. 3. The frequencies of CD4+CD25+CD127– T-cells in each group of subjects

In BPHF and BPCHD patients, B lymphocyte percentage and NK were all increased significantly in comparison to the BP group. After lower respiratory tract infection, B lymphocyte proliferated and differentiated rapidly. With the development and deterioration of the disease, CD19+ levels were increased gradually, and more and more NK cells were required in order to clear the pathogen and kill infected cells.

The differences of the frequencies of CD4+CD 25+CD127– T-cells in four groups were all not statistically significant. These findings suggested that the occurrence and development of heart disease might be not associated with the Tregs of the body.

Conclusions

In conclusion, comparing with the children without CHD, the cellular immunity function of the children with CHD was vulnerable to be destroyed when suffering from BP, which might be attributed to blood circulation difficulties.

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The authors declare no conflict of interest.

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