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Original Article

T Helper 2 Cytokine Analysis of Bronchoalveolar Lavage in the Murine Model of Allergic Broncho Pulmonary Aspergillosis

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Background and Aims: *Aspergillus fumigatus* is a sporadic fungus that causes different infections and allergies in immunocompromised patients. The allergic disease caused by this fungus is called allergic bronchopulmonary aspergillosis (ABPA). ABPA is considred important in atopic and immunocompromised individuals, which can result in inflammation and epithelial damage. Therefore, the aim of this study was to evaluate the T helper (Th)2 responses in a ABPA murine model by measuring the main cytokines involved in Th2.

Materials and Methods: Twenty male BALB/c mice were divided into two groups of 10 mice each: control and ABPA group. ABPA was induced by inhalation of *A. fumigatus* conidia intranasally. Total and specific IgE were measured in the mice sera. Levels of cytokines in broncho alveolar lavage (BAL) of under studied groups were measured by Enzyme-linked immunosorbent assay three weeks after the treatment.

Results: The obtained results indicated that total and specific IgE increased in the ABPA group ($p<0.05$). The levels of Interleukin (IL)-4, IL-5 and IL-13 in brocho alveolar lavage of ABPA group was significantly higher than the control group ($p<0.05$), whereas interferon-gamma levels did not reveal any significant differences between the studied groups.

Conclusions: The findings of the present study confirmed the role of Th2 cytokines in the ABPA reactions. However, more comprehensive studies are necessitated to determine the exact mechanisms of immune responses to ABPA as well as the role of Th1/Th2 responses in control of ABPA reactions. Regulation of Th2 responses could be regarded as a potential therapy for ABPA as well.

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Introduction

Aspergillus fumigatus is a sporadic fungi found in many environments [1, 2]. This fungus is responsible for multiple pulmonary diseases which indicates both the patient's immunological status and the pre-existing integrity of lungs [3]. Allergic bronchopulmonary aspergillosis (ABPA) is a kind of lung hypersensitivity induced by *Aspergillus fumigatus* that occurs in children and adults suffering from chronic lung diseases [4]. *Aspergillus* colonization in lungs results in an immune response to different antigens of *Aspergillus fumigatus* [5]. Such factors as genetic variations, activation of bronchial epithelial cells in asthma and cystic fibrosis affect development of $CD4^+$ T helper (Th)2 response activation and production of specific Immunoglobulin (Ig)E, IgG and IgA antibodies to *Aspergillus fumigatus* [6-8].

ABPA is observed in 2% of patients with asthma in the community setting and 28% in the referral setting [5]. The main symptoms of this disease entail asthma and activated Th2 cells, although IgG-mediated Arthus reaction [9] and autoimmune reactions [10] are involved in its pathogenesis as well. Main characteristics of ABPA consist of eosinophilia, pulmonary infiltration, central bronchiectasis, increase in serum IgE as well as specific IgE and IgG to *Aspergillus* [11]. This disease has been demonstrated as an immunological condition mainly diagnosed via late and immediate cutaneous reaction to *A. fumigatus* antigens, as well as increase in serum IgE and precipitating antibodies to *A. fumigatus* [12, 13]. The mechanism in which *A. fumigatus* interacts with the immune system is not thoroughly understood, though it is believed that Th2 cytokines production including Interleukin (IL)-4 and IL-5 are stimulated by *Aspergillus* antigens [11, 14]. Hence, the present study was carried out to evaluate the role of Th2 response in a murine model of experimental ABPA.

Materials and Methods

Twenty male BALB/c mice, aged 4-8 weeks, were purchased from Razi Vaccine and Serum Research Institute in Karaj, Iran. The mice were kept at 20ºC with relative humidity of 55±10% and 12hrs of light/dark cycles. The mice were divided into control and ABPA groups (n=10). All the mice were adapted to the experimental conditions one week prior to the examination. It is worth mentioning that all procedures involving animals and their care were conducted in conformity with the national as well as international laws and policies.

ABPA was induced by instilling the culture filtrate antigens of *Aspergillus fumigatus* into the nostrils of BALB/c mice through sterile micropipette tips. Sterile Phosphate-buffered saline was inoculated to the control group. Serum total IgE and specific IgE to *A.*

fumigatus allergens were evaluated as described by Kurup et al. [15, 16].

Three weeks after ABPA development, broncho alveolar lavage (BAL) was collected from the control and ABPA mice groups. The cytokines levels of BAL were measured using Enzyme-linked immunosorbent assay (ELISA). BAL supernatant of the mice was isolated by centrifugation of samples at 300 gr for 10 min. Th2 cytokines, IL-4, IL-5, IL-13 and Interferon-gamma (IFNγ) were assayed using ELISA method according to the manufacturer's instructions (eBiosciences, Austria). Briefly, a 96-well flat bottom plate was coated with capture antibody specific to each cytokine. The plate was washed and blocked before 100 µl of the supernatants. Serially diluted specific standards were added to the respective wells. Following a series of washing, the captured cytokine was detected using the specific conjugated detection antibody. The chromogen/substrate reagent was added into each well and, after color development, the plate was read at 450 nm using an ELISA plate reader.

Statistical Analysis

The study data were analyzed using SPSS software version 20 (SPSS Inc, Chicago, IL, USA). Kolmogrov-Smirnov test was utilized to evaluate the normality, and values were examined by the independent t-test to detect the differences between the groups. Moreover, the study data were demonstrared as Mean±SEM and a P-value of less than 0.05 was considered significant.

Results

Total IgE levels had elevated in mice sera involved with ABPA and this difference was reported to be significant between the study groups ($p<0.05$). Although specific IgE to *A.fumigatus* conidia increased significantly in the ABPA group, no specific IgE was detected in sera of the control mice. As it is depicted in Fig.1, Cytokines measurements in BAL of ABPA and control groups demonstrated that Th2 cytokines increase greatly in ABPA compared with the normal mice ($p<0.05$). IL-13 had the highest increased levels among the studied cytokines, whilst IL-5 increase was the lowest in comparison with the control group (Fig.2A, B, and C). In contrast, no significant changes were observed between ABPA and control groups ($p>0.05$) in regard with levels of IFNγ , though a slight reduction was observed in IFNγ secretions in BAL of ABPA mice (Fig.2D).

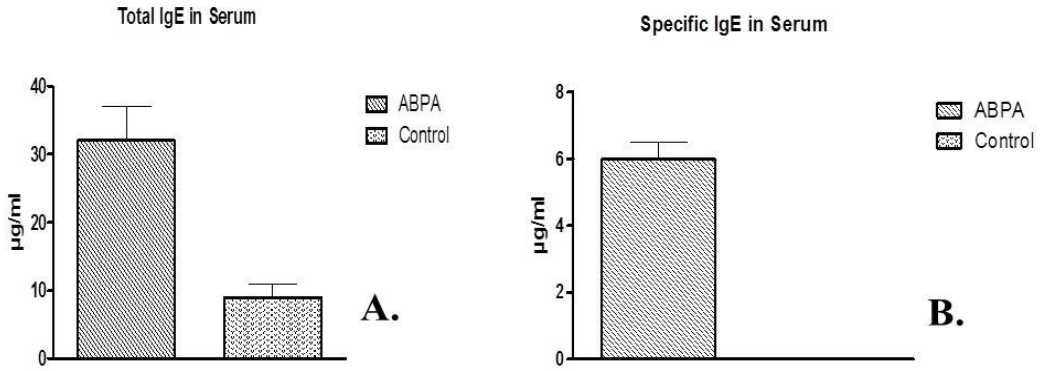

Fig.1. Total and specific IgE in sera of ABPA and control groups are compared. **A.** levels of total IgE (µg/ml) in control and ABPA groups are shown. **B.** Levels of specific IgE to *A. fumigatus* conidia in study groups are compared. As it is observed no specific IgE level was detected in the control mice.

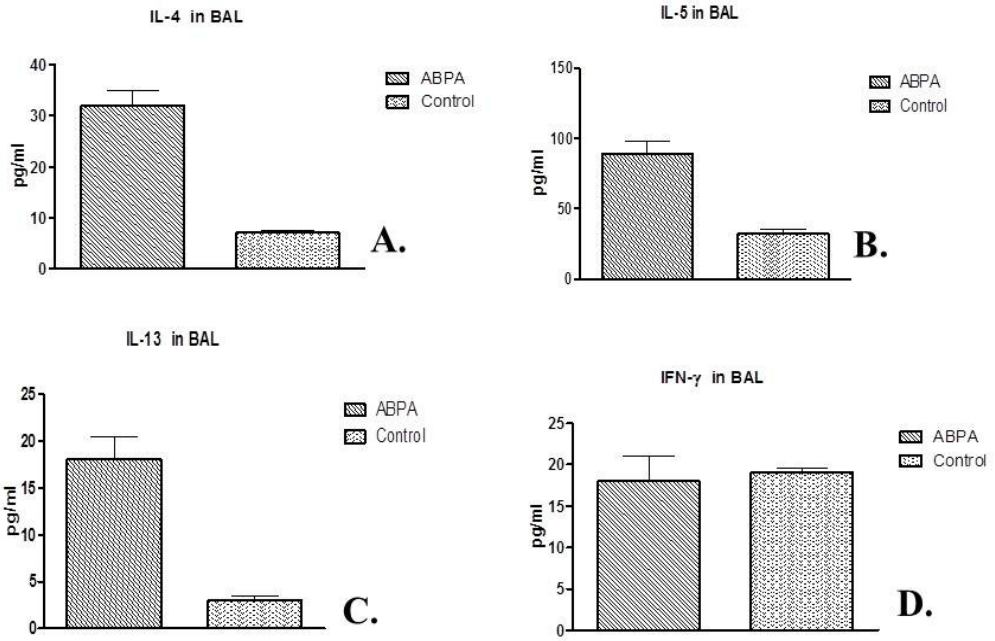

Fig.2. Levels of Th2 cytokines (pg/ml) are demonstrated. **A.** IL-4 significantly increased in the BAL of ABPA infected mice compared to the control group. **B.** IL-5 levels increased significantly three weeks after *A. fumigatus* exposure compared to the control group. **C.** IL-13 had the highest increased rates among under study cytokines in BAL compared to the normal cytokines levels in the control group. **D.** IFNγ levels had insignificantly decreased in the BAL of ABPA mice than the control group mice ($p>0.05$).

Discussion

Aspergillus fumigatus is a ubiquitous fungus that doesn't affect immunocompetent and nonallergic individuals. However, it owns the potentiality to cause several diseases including life threatening infections in immunocompromised and atopic patients [3,17-19]. Allergic diseases of *A. fumigatus* are referred to as ABPA [20]. Persistence of *Aspergillus* conidia and hyphae in lungs provokes immune responses [21]. The systemic immune response in ABPA is

distinguished by changes in IgE, IgG and Th2 cytokine profiles [22-24].

In the current study, ABPA was induced in the mice by administration of *A. fumigatus* conidia intranasally. Mice sensitivity was confirmed by measuring the total IgE and specific IgE. IL-4, IL-5, IL-13 and IFNγ were assayed in the mice BAL in order to evaluate Th2 response in ABPA. As a matter of fact, role of IL-4 in allergic reactions is well recognized. It is well documented that this cytokine induces Th2 response and activates B cells in order to produce IgE [25]. Several studies have shown that patients with ABPA produce significantly more amounts of IL-4 and IL-5 by T cells in response to *A. fumigatus* antigens than IFNγ [26, 27]. Same results have been found in patients with cystic fibrosis [28], which these findings are in consistence with the results of the present study. A significant increase was observed in IL-4 and IL-5 BAL levels as well. It is believed that this immune response is activated by Asp f1 and Asp f2 antigens [13, 26]. Interestingly, B cells from ABPA individuals are more sensitive to IL-4 stimulation in comparison with atopic and non-atopic controls; which has been documented by up-regulation of CD23 and CD86 [29]. Treatment of ABPA mice with anti IL-4 antibody or exposure of IL-$4^{-/-}$ mice to *A. fumigatus* conidia discloses the significant role of IL-4 in development as well as maintenance of IgE, eosinophilia and airway hyper responsiveness in ABPA models [30-34]. Some studies on IL-5 role in ABPA development have displayed a limited action for IL-5 [30, 32, 35, 36]. Another recent study, carried out on gene expression profiles during ABPA, demonstrated over expression of Th2 associated genes like IL-4 and IL-5 receptor genes [37]. These findings conclude that an increase in secretion of Th2 cytokines is regarded as an important immune weakness in ABPA; however, the role of this response in progression of ABPA is yet to be understood.

Moreover, a recent study on the role of IL-10 in allergic responses to *A. fumigatus* antigens in IL-$10^{-/-}$ mice revealed that in absence of IL-10, numerous amounts of IL-4, IL-5 and IFNγ are produced. These mice were reported to have mortality rates 50-60% greater than the wild type mice [38]. In this study, IL-10 levels were not measured although IL-5 and IL-4 levels had increased significantly, no relation was observed between amounts of Th2 cytokines and mortality rates.

IL-13 is another cytokine of Th2 response that is very similar to IL-4. IL-13 and IL-4 both induce antibody class switch to IgE production in B lymphocytes [7]. It was demonstrated that following IL-13 stimulation in ABPA Cystic fibrosis patients no significant increase was observed in CD23 and CD86 expression by B cells compared to the control patients [25]. Recently, this cytokine has been studied in cases of chronic *Aspergillus* induced allergic reactions. It has been suggested that this cytokine is responsible for inflammatory response, hyper reactivity and remodeling of airways [31, 39, 40]. In addition, studies on IL-13 neutralized mice revealed that this does not prevent clearance of *A. fumigatus* conidia in *Aspergillus* sensitive mice. In the present study, IL-13 levels in BAL of ABPA mice had

increased indicating the significance of IL-13 in allergic reactions to *A.* fumigatus [41]. Identification of genes involved in allergic aspergillosis can be regarded as another approach in order to better understand the mechanism of the disease.

Conclusion

Recent data indicates that the risk of fungus related allergic diseases is growing as a result of industrialized life. Therefore, it is important to understand the immune responses to *A. fumigatus* allergens in more details. This study emphasizes the participation of Th2 cytokines in allergic reactions elicited by *A. fumigatus*, which displays that clinical treatments for ABPA may best regulate the production and/or actions of these Th2 components in the lung. As a result, drugs targeting IL-4, IL-13 and their receptors could serve as potential treatments in regard with ABPA therapy.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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