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ORIGINAL ARTICLE

Alternaria alternata acts on human Monocyte-derived Dendritic cells to mediate Th2/Th17 polarisation

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KEYWORDS
Alternaria alternata;
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Abstract
Introduction: Although the mechanism of asthma is not precisely understood in humans, clinical and epidemiological studies have offered a potential relationship between exposure to environmental fungi, such as *Alternaria alternata* (*A. alternata*) and the development and exacerbation of asthma. The aim of this project is to investigate the mechanisms of Th2 responses by *A. alternata* as a clinically relevant model for the environmental exposure.
Materials and methods: Plastic adherent monocytes were cultured with granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) to convert these cells into Monocyte-derived Dendritic cells (MoDc) and then matured in the presence of Monocyte-Conditioned Medium (MCM) as the control group and MCM+ *A. alternata* extract as the inductive groups.
Results: The results indicated that the expression of CD14 decreased and CD83 and anti-human leukocyte antigen-DR (HLA-DR) increased in the inductive groups in comparison with the control group. More importantly, *A. alternata* inhibited IL-12 production by activated dendritic cells (DCs), and the DCs exposed to *A. alternata* enhanced the Th2 polarisation of $CD4^+$ T cells. The production amount of IL-10 overcame IL-12 as well as Il-23 increased significantly, and hand in T cells the production of cytokines Interferon-γ (IFN-γ) decreased. However, both IL-17 and IL-4 increased ($p < 0.05$). Phagocytic activity in the inductive groups decreased significantly compared with the control group.
Conclusion: The asthma-related environmental fungus *A. alternata*, with an effect on dendritic cells profile mediates TH2/TH17. Such immunodysregulation properties of causative environmental fungi may explain their strong relationship with human asthma and allergic diseases.

Abbreviations: *A. alternata*, *Alternaria alternata*; CD, cluster of differentiation; DCs, dendritic cells; FACS, fluorescence-activated cell sorting; FBS, Foetal Bovine Serum; GM-CSF, granulocyte macrophage colony stimulating factor; HLA-DR, anti-human leukocyte antigen-DR; IFN-γ, Interferon-γ; IL, interleukin; ImDC, immature dendritic cell; MCM, Monocyte-Conditioned Medium; mDC, mature dendritic cells; MFI, mean fluorescence intensity; MoDc, Monocyte-derived Dendritic cell; PBMC, Peripheral Blood Mononuclear Cell; Th, T-helper; Treg, T-regulatory.

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Introduction

Asthma exacerbation is a major agent of disease for the patients involved with moderate asthma. Sensitisation and exposure to the fungal allergen *Alternaria alternata* (*A. alternata*) are a risk factor for the start of severe asthma symptoms, including threatening and mortal responses.[1–3] The exclusive relation between *A. alternata* and exacerbation of asthma is clear; but the aetiology of the exclusive pathogenesis of *A. alternata* has not been precisely understood. These responses are mediated by CD4-derived T-cells that are polarised to Th2 or Th17cells phenotypes.[4] Dendritic cells (DCs) are one of the main cellular members regulating immune tolerance and response.[5–7] DCs by producing various secretory substance or membrane ligand determine the eventuality of T cell responses, such as T-helper1 (Th1), T-helper2 (Th2), or T-regulatory (Treg) responses.[8] Currently, limited data exist concerning how asthma patients develop such disturbance Th2 immune responses to environmental allergens. Overall, dendritic cell pulse for an innocuous antigen or medium is considered a tolerogenic occurrence.[5,9] On the other hand, the mechanism of Th2 immune responses may reflect Th2 immune responses to parasite infection. In specific terms, asthma may offer overexpression of immune responses to chitin-containing organisms, specially mites, and fungi.[10,11] However, the association of immunological mechanisms with impulsive due to chitin-containing organisms and development of Th2 immune responses are not fully understood. The aetiology of human asthma is complicated and multifactorial; probably it involves the interference between genetic factors and environmental motives. As a major environmental motive, the relationship between fungal exposure and asthma has been recognised pathologically and epidemiologically.[1] In particular, there is a great deal of evidence suggesting a relationship between a ubiquitous environmental fungus *A. alternata* and asthma.[2,12] *A. alternata* is ubiquitous and unique both in outdoor and indoor,[13] and for the high rates of spore germination and antigen propagation.[14] Exposure to *A. alternata* is a major risk factor for asthma and allergic.[15] Severe asthma and acute exacerbations of asthma have also been associated with increased airborne exposure to *A. alternata* spores and consequently its pollen pollution.[16] In mice, the relationship between asthma and stimulation by fungal antigen, such as aspergillus and *A. alternata*, has been clinically and pathologically explained.[1,2] The mouse models of asthma offer a wide range of experimental possibilities, but due to their limitations such as their different physiology, we decided to use other models.[17] To survey the immunological mechanisms involved in Th2 responses, we used *A. alternata* to model environmental exposure correlated to asthma.

Materials and methods

The objective of this study was to perceive the mechanisms engaged in asthma. The study was conducted in the cleaning room of the research centre of biotechnology at Urmia University and zoonosis researches central of Jahrom Universty of medical sciences from September 2013 to February 2014. Various tests have been used to evaluate Autologous T cell responses by co-culturing with DCs. In the meantime, the phagocytic activity of pulsed DCs by *A. alternata* was examined. These experiments were replicated five times.

Preparation of fungus extract

A. alternata spore purchased from Iranian industrial and scientific research organisation (PTCC 5248) was cultured on Sabaroud dextrose agar at 25 °C for five days. Mature fungi subculture on Czapek's agar was used to produce a large number of spores. In addition, the amount of 1×10^7 spores was collected by Hanks solution and passed through sterile Tampon, subsequently transported on liquid culture of Yeast nitrogen base (37 °C, 5% CO_2, 5% humidity) for 48 h in order to improve the growth of mycelium. Then grown-up centrifuged myceliums ($2000 \times g$) were added to PBS buffer containing 2×10^{-3} M protease inhibitor (Sigma-USA), 50×10^{-3} M EDTA (Sigma-USA), 50×10^{-3} Tris–HCl and sonicated on a sonicator (20,000 AMP), 10 s interval (apparatus 10 s was off and 10 s was on) and totally 5 min duration. After sonication, the homogenised fungi were centrifuged ($7000 \times g$ at 4 °C), and the supernatant were dialysed by dialysing tube (cut off: 14,000) full off. The extract was dehydrated by freeze dryer (CHRIST ALPHA 1.4, UK) to reduce the volume of water.[18] The resulted extract was filtered through fine pores (22 μm in diameter) and protein of solution was measured by BRADFORD method.[19] The final extract was considered 1 mg/ml and it was stored at −70 °C until use.

Preparation of Peripheral Blood Mononuclear Cell (PBMC)

Heparinised blood was obtained from volunteer donors (200 U/ml) in sterile conditions and mixed with the equal volume of culture medium RPMI-1640 (Gibco, UK). Diluents blood was transported gently on Ficoll-Hypaque (Sigma, USA) and centrifuged for 15 min in $800 \times g$. PBMC located between Ficoll-Hypaque and diluents blood were collected, and washed by RPMI-1640 then it was centrifuged for 10 min in $480 \times g$ in order to be deleted from platelets. Cellular pellet was washed again by RPMI-1640 for 10 min in $200 \times g$. The number and viability of cells were assigned by Trypan blue.[20]

Production of control DCs in the presence of Monocyte-conditioned Medium (MCM) and its Inductive with *A. alternata* extracts

MCM was prepared as described elsewhere. Briefly,[20] PBMC was plated onto the human Ig-coated Petri dish for one hour, non-adherent cells were washed away and Ig-adherent cells were incubated in fresh complete medium with 1% autologous plasma at 37 °C for 24 h. The medium was collected and saved at −20 °C until time of use. The isolation of T cells was performed using anti-CD3 magnetic bead cell sorting technique (Miltenyi Biotec, Germany). 4×10^6 PBMC in RPMI-1640 (6 ml) were transferred to T-25 flask and after 2 h the adhered cells to the T-25 flask were washed three times with RPMI-1640 and finally the adhered cells cultured with

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