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# INDUCTION OF PULMONARY ALLERGIC RESPONSE IN RABBITS BY THE INTRANASAL CHALLENGE WITH PENICILLIUM DIGITATUM SPORES

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## ABSTRACT

Background: Penicillium digitatum are the major indoor and outdoor fungi considered as a respiratory pathogens when acquired through inhalation of the spores. when Cell-mediated immunity (CMI), encompassing cells of the innate and adaptive immune systems become impaired through a widespread use of the antibiotics or drugs and in immunocompromised patients, such spores may germinate and proliferate in the host, and toxicity may arise, leading to various clinical outcomes like pulmonary allergic diseases Materials and methods: Rabbits were orally administrated with Cefoperazone antibiotic for 10 days, antibiotic was replaced with sterile normal saline in control group. For intranasal challenge of rabbits the fungal inoculum at $10^7$ conidia / rabbit concentration were weekly given for 6 weeks. Results: the exposed rabbits to the P. digitatum conidia in presence of antibiotic developed Th2- mediated allergic response as indicated by histological examination of lung which explain hypersensitivity pneumonitis and an elevation of the serum IL-4, IgE and IgG levels. Conclusion: Inhalation of P.digitatum conidia stimulates Th2–mediated plumonary allergic response following an antibiotic administration. Increased level of serum IgG represent as a interest indicator for development hypersensitivity pneumonitis.

**KEYWORDS:** Penicillium Digitatum; Pulmonary Allergic Response; Hypersensitivity Pneumonitis

## 1. INTRODUCTION

The atmosphere is filled with thousands of fungal spores (conidia) exceed $10^9$ per cubic meter. They originate from more than a hundred fungal species belonging mainly to the genera Cladosporium, Penicillium, Alternaria and Aspergillus (Codina et al ,2008). Although these conidia contain many antigens and allergens do not activate the host innate immune cells continuously and do not induce detrimental inflammatory responses following their inhalation. Thus, airway exposure to allergens lead to tolerance rather than sensitization

without prior systemic sensitization in immunocompetent individuals (Aimanianda et al, 2009; Ooij, 2009)Noverr et al (2004). Have developed a mouse model of allergic airway disease that resulted from antibiotic therapy and microbiota disruption as attempt for immune modulation

Viable microorganisms such as spores of fungi and actinomycetes may be able to germinate and proliferate in the host when the host's defence systems fail (Cell- Mediated Immunity CMI become impaired in some individuals, like over drugs taker and immunocompromised patients) toxicity may arise, leading to various clinical outcomes such as organic dust toxic syndrome (ODTS), mucosal membrane irritation, and chronic restrictive and obstructive lung diseases such as hypersensitivity pneumonitis and allergic bronchopulmonary aspergillosis (ABPA). (Gauthier and Klein, 2008; Eduard, 2009).

The pathogenesis of hypersensitivity pneumonitis includes a mixture of immune complex (type III) and cell-mediated (delayed, type IV) hypersensitivity responses. It seems to be more important in populations occupationally exposed to . ABPA is an inflammatory disease in which fungi (usually Aspergillus fumigatus) grow in mucous secretions in the lung. ABPA is thought to involve type I and type III hypersensitivities associated with IgE- and IgG-mediated immune response to Aspergillus species fungi .( Horner et al, 1995; Eduard, 2009).

In the lung, after exposure to fungal spores , the alveolar macrophages are the first line of innate cellular defense against fungal infection, they attach inhaled spores by toll-like receptors (TLR) and killed them, and if spores withstood macrophage attack, germination was prevented (Romani, 2004; Blanco and Garcia, 2009). The second defense line in lung against a fungal infection is adaptive immune response associated with role dendritic cells that phagocytise pathogens and present their antigens to precursor T-helper (Th) cells, which may mature into Th1 and Th2 cells (Bozza et al, 2002).. The Th1 cells mediate a cellular response by stimulating macrophages to phagocytise the pathogen and T cells to develop into cytotoxic T cells that kill specific organisms. The Th2 cells stimulate B cells to proliferate into specific antibody-producing plasma cells. Dendritic cells produced IL-12 and TNFα after ingestion of spores, which primed the maturation of precursor Th cells to gamma interferon (IFNγ)-producing Th1 cells, whereas ingestion of hyphae induced IL-4, IL-10, and TNFα production and priming of IL-4–producing Th2 cells (Shoham and Levitz2, 2005; Eduard, 2009). The Th1 and Th2 cells further modulate the type of antibodies that are produced by secretion of different cytokines. Th1 cells promote the development of IgG antibodies that bind specifically to antigens and facilitate their uptake by phagocytic cells. Th2 cells may

stimulate B cells to produce IgE antibodies and can also induce proliferation of mast cells and eosinophilic leukocytes, which are important characteristics of the allergic response (Bellocchio, et al ,2005). Spores of Penicillium spp. do occur outdoors but are far more important as indoor fungal contaminants. Bronchial challenges with Penicillium sp. spores induced immediate- and delayed-type asthma in sensitized subjects (.( Horner et al, 1995; Eduard, 2009). So the objective of the present study is to select Penicillium digitatum spores and to investigate whether they can be develop airway allergic (Th2) response following antibiotics administration in the rabbit model

## 2. Materials and methods

**2.1. Laboratory animals**: twenty fife NewZeland white rabbits (1.5- 1.75 kg) were purchased from College of Science laboratories of Babylon University, and housed in specific pathogen-free conditions. They were given a commercial cow food with free access of water

**2.2. Antibiotic treatment**: Fifteen animals were orally administrated with Cefoperazone (0.5 mg/ml, Sigma-Aldrich) for 10 days to allow gastrointestinal (GI) bacterial flora disruption and to grow GI yeast like candida albicans opportunists. The control animals were replaced orally administrated with sterile normal saline (Noverr et al, 2004).

**2.3. Intranasal challenge with P.digitatum spores:** P. digitatum fungus was grown in PDA for 14 days. Spores were harvested by washing plates with sterile 0.1% Tween the resulting fungal suspension then filtered through two layers of sterile gauze to remove hyphae (Noverr et al, 2004; Noverr et al, 2005). For infection, the conidia were twice washed with phosphate buffer saline (PBS) at 3500 r/m for 5 minutes, the supernatant was discharged and the pellet (conidia) counted by using W.H.O Opacimeter to $10^8$ conidia/ml in PBS then diluted to $10^7$ conidia / rabbit in a 1 ml volume. Rabbits were weekly given a 0.5-ml of P. digitatum conidial suspension in each nostril for 6 weeks.

**2.4. Histological examination:** All the animals were autopsied after 48 hours of the final conidial challenge, where the lungs and spleens were removed, washed with normal saline then placed in tubes containing 5 ml formal saline for histopathological analysis

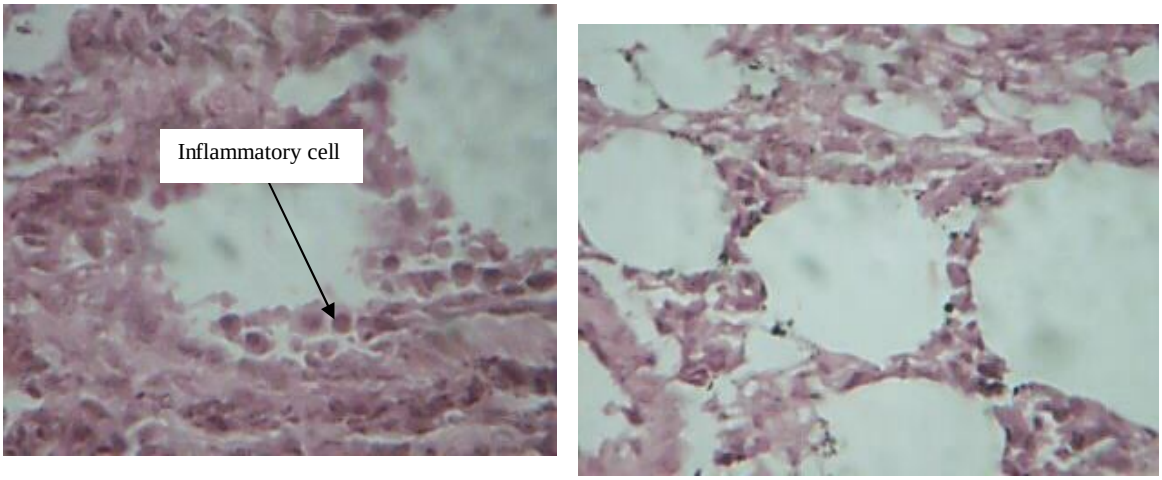
**2.5. Serological examination**: Serum IL-4, and total IgE and IgG concentration levels were measured by ELISA according to the instructions of the manufactured companies ( Cusabio Biotech Co.,LTD; InterMedical Diagnostics.it and Diagnostic Automation , INC.) respectively

**2.6. Statistical analysis:** All analyses were conducted using SPSS 18.0 The Student's t test (two-tailed, unequal variance) was chose to analyze the significance of differences between experimental groups. Data with a P value of $\leq 0.05$ was considered to be significant

## 3. Results

### 3.1. Histological Investigation

The histological analysis of rabbits lungs demonstrated that P.digitatum conidia were able to induce hypersensitivity pneumonitis in presence of the antibiotic administration (Figure 1, A), while in the untreated animals had not develop detectable histopathological changes in the lung sections (Figure 1, B)




A B

Figure-1: A: hypersensitivity pneumonitis. High power view of lung biopsy specimen of the antibiotic treated and P.digitatum conidia challenged rabbit: shows interstitial mononuclear cells and lymphocytes infiltrate that restricted to the alveolar wall B: normal lung tissue without histopathological changes of the control rabbits (H and E, ×400)

### 3.1. Serological Investigation

#### 3.1.1 Total IgE ELISA

The antibiotic-treated rabbits and exposed to P.digitatum conidia developed airway allergic response as indicated by a significant increase serum IgE concentration , it was above 35.7 IU/ml compared with the untreated animals and exposed to the fungal conidia which showed that normal value of serum IgE level . The means were 42.45 IU/ml and 30.24 IU/ml respectively (Figure 2, A) . $P \leq 0.05$

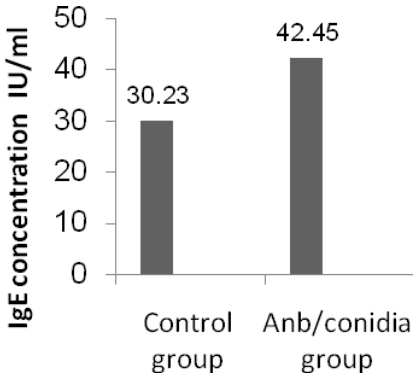
#### 3.1.2. Total IgG ELISA

Serum IgG level was also elevated in Intranasal P.digitatum conidia -challenged animals which treated with antibiotic, the concentration was above 0.31 mg/ml compare with untreated animals which had a normal value of serum IgG concentration, the means were 0.86 mg/ml and 0.22 mg/ml respectively (Figure- 2,B). $P \leq 0.05$.

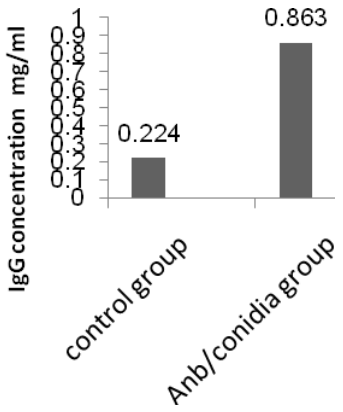
#### 3.1.3. IL-4 ELISA

An exposure of the rabbits to P.digitatum spores in presence of antibiotics produced a significant elevation of IL-4 concentration in the serum , the concentration was above 16 pg/ml , the mean was 20.57 pg/ml compared with conidial exposure of the rabbits in absence of antibiotics, the mean was 8.33 pg/ml (Figure-2, C). $P \leq 0.05$

A



B




C

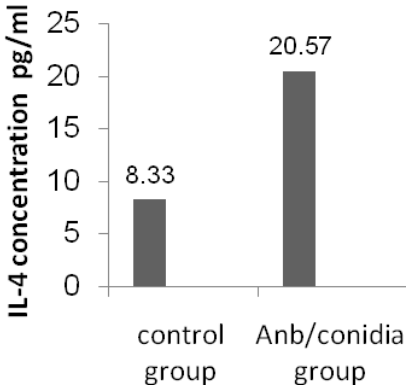



Figure-2: serum IgE ,IgG and IL-4 concentration measured by ELISA . Results were expressed as a means ± standard error. Control group = untreated animals and challenged with P.digitatum conidia, Anb/conidia group= treated animals with antibiotic and challenged with P.digitatum conidia. P≤0.05

**4. Discussion**

The main exposure route of fungal spores is by inhalation. Aimanianda1 et al (2009) and Bayry et al (2010) documented that Although these conidia contain many antigens and allergens do not induce inflammatory responses following their inhalation and explained that returns to the presence of the surface layer called rodlet layer composed of hydrophobic RodA protein on the dormant conidia which did not induce dendritic cell or alveolar macrophage maturation and activation, and failed to activate helper T-cell immune responses in vivo. Noverr and his colleagues (2004 and 2005) succeed to demonstrate one aspect of the

hygiene hypothesis which refer to widespread of antibiotic therapy and alterations in microbiota populations, including growth of C. albicans can alter immune responses on the mucosa via the production of prostaglandin-like oxylipins which can inhibit Th1-type immune responses and promote Th2-type responses (Matsuoka et al, 2000), leading to allergic responses to inhaled fungal elements, to prove this hypothesis they introduce a mouse model and A.fumigatus conidia an allergen.

The present study shed light on the capacity of P.digitatum which are not recoded by recent publications to develop airway allergic disease.

Our result demonstrated that the treated and P.digitatum conidia- challenged Rabbits developed hypersensitivity pneumonitis and increased numbers of inflammatory cells in lung tissue, accompanied with elevation of serum IL-4, IgE and IgG levels. These data referred to P.digitatum conidia exposure could elicit Th2 -mediated allergic response. Th2cells produce predominantly cytokines such as interleukin 4 and tend to promote antibody production (IgE and IgG1)

Hypersensitivity pneumonitis (HP) is a diffuse granulomatous interstitial lung disease caused by inhalation of various antigenic organic particles (Mohr, 2004; Silva et al, 2005). Eduard (2009) documented that hypersensitivity pneumonitis (HP) is an allergic disease, although the mechanisms are not entirely clear. The disease is not IgE mediated, as IgE antibodies are not generally found in the patients. Specific IgG antibodies to fungi can often be demonstrated in serum of patients, indicating a type III allergic reaction. However, the role of IgG antibodies in the disease is unclear, as these antibodies are common in healthy exposed individuals. The presence of serum IgG antibodies is therefore regarded as a marker of exposure and not of disease.

Similar findings were recorded by Hogaboam et al (2000) and Havaux et al (2005) the first auther observed that increased total serum IgE accompanied with increased IL-4 in the lung tissue of a mouse exposed to Aspergillius fumigatus. While Havaux et al (2005)developed experimental airway allergic disease in a sensitized mouse by Alternaria alternata intranasal exposure and demonstrated that increased nutrophils and eosinophils were paralleled with increased IL-4 and IL-13.

## 5. Conclusion

Inhalation of P.digitatum conidia stimulates Th2–mediated plumonary allergic response following an antibiotic administration. Increased levels of each eosinophils and neutrophils in the lung tissue, and the serum interleukin 4 (IL-4) and immunoglobulin E (IgE), characterize the allergic response. Increased level of serum IgG represent as a interest indicator for development hypersensitivity pneumonitis.

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