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Allergenic and Pathophysiology Effects of Aspergillus Niger Spore Protein

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Abstract
Aspergillus niger is among the most dominant and virulent fungal species preponderant in both indoors and outdoors environment. The allergenic potential of Aspergillus niger spores protein was studied in mice in other to elucidate their possible clinical effects in human. Spore protein was extracted using phosphate buffered saline (pH7.4). Mice were exposed to Aspergillus niger allergen by two subcutaneous and one intranasal injections, weekly for four weeks. Results revealed a significant rise in immunoglobulin E antibodies (IgE) level at first sensitization only. Immune cells such as lymphocyte, neutrophil, ring neutrophil and monocyte differed significantly from control. They were an increase in ring neutrophilic response which was associated with infiltration of inflammatory cell in the respiratory organ of the group sensitized with Aspergillus spore allergen. The allergen penetrated vital organs such as kidney and liver and caused structural and cellular alterations. There were evidences of degeneration of glomeruli and damages of the basement membrane of the bowmans capsule and collecting tubules in the kidney. There were also mild infiltration of inflammatory cells within the parenchyma particularly in the sinusoid of the hepatic tissue of the liver. Results revealed that Aspergillus niger is allergenic candidate with potential of causing pathologies on the respiratory and vital organs.

Keywords: *Aspergillus niger;, allergy, pathologies, mice, immune cells*

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I. Introduction

Fungi are found in diverse environment, most of them are usually preponderant outdoors during rainy periods, as rain favours their sporulation. In indoors environment, wetness and poor ventilation favour their sporulation and release of abundant spores into ambient air. Also they are usually laden in dust particles in an enclosure, on rugs, carpets etc and most abundant in organic matter (Ashok, 2004). Many studies have shown that indoor *Aspergillus* is an important human health risk for those who are susceptible to fungal infection, allergy and asthmatics etc (Ashok, 2004; Denning *et al.*, 2014).

Aspergillus niger is a mold, this means a group of fungi in which the growth of hyphae results in discoloration and a fuzzy appearance. It belong to division of Ascomycota and class Eurotiomycetes. Fungi belonging to the Ascomycota as well as to the Basidiomycota have been known to cause a broad panel of human disorders for decades. They may live as saprophytes, parasites or symbionts of animals and plants in indoor as well as outdoor environment. In contrast to pollen, fungal spores and/or mycelial cells may not only cause type I allergy, the most prevalent disease caused by molds, but also a large number of other illnesses, including allergic bronchopulmonary mycoses, allergic sinusitis, hypersensitivity pneumonitis and atopic dermatitis; and, again in contrast to pollen-derived allergies, fungal allergies are frequently linked with allergic asthma (Denning *et al.*, 2014). Sensitization to molds has been reported in up to 80% of asthmatic patients. (Simon –Nobbe et al., 2008) Exposure to *Aspergillus* has been reported to also cause several types of human health problems, primarily irritations, infections, allergies, and toxic effects, and it has been suggested that toxigenic *Aspergillus* are the cause of additional adverse health effects (Hedayati *et al.* 2007; McGinnis 2004; Denning 1998). *Aspergillus* can also cause infection, such as invasive aspergillosis and sinusitis, typically in individuals with compromised immune systems (Hedayati *et al.* 2007). To the extent that fungi are involved in these processes, the inhalation or ingestion of fungal cellular debris is thought to be the principal route of exposure.

Aspergillus do not only cause various allergic reactions such as rhinitis, conjunctivitis, asthma exacerbations etc, but they also produce mycotoxins which affect the immune system. Mycotoxins are non-volatile, secondary metabolites of low molecular weight produced by fungi which impair the immune system and have neurotoxic, mutagenic, carcinogenic and teratogenic effects. Diseases caused by mycotoxins are called mycotoxicoses. The severity of toxic effects depends on the type of mycotoxin, the duration and dose of exposure and the age, health and nutritional status of the individual affected (Simon –Nobbe *et al.*, 2008). Mycotoxins may occur in spores, mycelia, and the matrix in which fungi grow. They constitute a huge health risk for farm workers, for persons living in houses with excessive mold growth and for persons exposed to

moldy material at the workplace (Simon –Nobbe *et al.*, 2008). The potential of *Aspergillus* species to produce highly diversified complex biomolecules such as multifunctional proteins (allergens, antigens, enzymes) and polyketides is fascinating and demands greater insight into the understanding of these fungal species (Preeti et al.,2011).

The genus *Aspergillus* is one of the most important filamentous fungal genera. *Aspergillus* species are used in the fermentation industry, some species are also responsible of various plant and food secondary rot, with the consequence of possible accumulation of mycotoxins. The aflatoxin producing *A. flavus* and *A. parasiticus,* and ochratoxinogenic *A. niger, A. ochraceus* and *A. carbonarius* species are frequently encountered in agricultural products. Studies on the biodiversity of toxigenic *Aspergillus* species is useful to clarify molecular, ecological and biochemical characteristics of the different species in relation to their different adaptation to environmental and geographical conditions, and to their potential.

Aspergillus spp. have been known to be one of the most prevalent airborne fungi in indoor environment. Indoor fungi are a mixture of those growing indoors and those that have entered from outdoors. Their incidence is influenced by humidity, ventilation, the content of biologically degradable material, and the presence of pets, plants and carpets (Simon –Nobbe et al., 2008). Predominant indoors fungi include *Scopulariopsis brevicaulis* , *Rhizopus* sp. (Vegas *et al.*,2012). Preponderant outdoors fungi include *Nigrospora, Puccinia, Dactylaria, Dreschlera, Venturia, Helminthosporium, Pithomyces, Ovularia, Alternaria*, Smut, *Curvularia, Cercosporella, Cladosporium, Stachybotrys* (Shelton et al.,2002;Ezike *et al*., 2016; Ezikanyi *et al* 2017; Ezikanyi and Sakwari 2018).

Exposure and sensitization to other fungal allergens can promote the development and worsening of allergic diseases. Although numerous species of fungi have been associated with allergic diseases in the literature, the significance of fungi from the genera *Alternaria, Cladosporium, Penicillium, Aspergillus,* and *Malassezia* has been well documented (Fukutomi and Taniguchi, 2015). However, it should be emphasized that the contribution of different fungal allergens to allergic diseases is not identical, but species-specific and depend on predisposition of the subject.

Humans inhale viable and nonviable fungi or their components in many indoor and outdoor environments, and mold-related exposures can pose a significant concern to human health Although a number of federal agencies provide guidance to the public on health effects associated with mold exposure and on ways to mitigate it, the United States Government Accountability Office (US GAO) reported a lack of federally accepted health-based standards for safe mold levels (Pandey *et al*,,2013). In Nigeria and other African countries, there are no information system on mold abundance in the air for early signs signals and informed adaptive strategies, also the allergenic potentials of most pollen and spores are not known.

II. Materials and Method

Pure culture media of *Aspergillus niger* were obtained from University of Lagos, Akoka, Nigeria. They were scooped out of the petri dishes, 50 g were measured using a sensitive weighing balance and defatted using diethyl ether for 2 times. They were extracted in 100 ml of 0.02 M phosphate buffered saline (PBS) at pH 7.4. The mixtures were stirred for 3 hours at 4 ^{o}C, filtered with a muslin cloth, centrifuged and supernatant retained. The protein precipitation was carried out with 50 g of ammonium sulphate and dialysed against PBS overnight. Protein concentration was assayed according to Bradford procedures. The crude spore protein were stored at -80 ^{o} C for later use in inoculating into experimental animals (albino mice). Albino mice which were 4-6 weeks were purchased from Lagos University Teaching Hospital (LUTH). Mice were fed ad libitum with a pelleted mouse diet and water. All experimental procedures conformed to international standard of animal welfare and ethical approval obtained from Ministry of Health, Ebonyi State. Crude spores extract (100 µ l) was inoculated into mice by two subcutaneous and one intranasal injections for four weeks. Blood samples were obtained by retro-orbital bleeding using heparinized capillary tubes and both pre and post sera obtained from the blood were stored at -80 ^{o}C for later use in detecting IgE levels using immuno assay. At the end of 4th week mice were sacrificed by cervical dislocation. The respiratory organ were obtained and processing of tissue samples for histological assessment followed established procedures. Blood smears were obtained from the tail, the thin blood smears were fixed with methanol for 2-3 mins. One in three dilutions of Leishman stain and buffered water was prepared and covered the slides for 7-10 mins. The stain was washed off in a stream of buffered water. Distillled water was added on a slide and left for 2-3 minutes to differentiate the film. The slides were allowed to dry on a rack. Using light Olympus CH Trinocular microscope (LM) fitted with Future WinJoe camera, blood smears were examined and the immune cells were identified and quantified on differential count.. Immuno assay for determination of IgE in mice sera were carried out using IgE kits purchased from AvivaSystems following manufacturers procedures.

III. Results And Discussion

Among all immune cells, neutrophils were dominantly recruited by the *Aspergillus niger* spores protein allergen (Table 1 and 2). They were presumably responsible for the recruitment of inflammatory cells in the lung of mice (Fig. 1). They were reported as the first cells recruited to the site of allergic reaction (Leiby *et al*.,1994). Their presence has been linked to inflammation and severe asthma (Rosales 2018; *Syabbal,2020*). Neutrophil was also discovered to play duplicitous function of inflammation and perhaps healing process (Butterfield *et al.*, 2006). Previous studies recognized that neutrophil contribute in the pathogenesis of a number of human diseases such as chronic obstructive pulmonary disease, Behlets disese and inflammatory arthritis (Butterfield *et al*, 2007). Evidence resulting from experiment with animal model shows that neutophils can contribute to progression of arthritis (Wright *et al*., 2010). In this study, recruitment of inflammatory cells were linked with chronic cellular inflammation and remodeling of the airways. Features such as this, contributes to asthma. Eynott *et al*., (2009) stated that inflammation, remodeling of airways in addition to subepithelial fibrosis and airway smooth muscle cell hyperplasia are features of asthma.

Immunoglobulin E antibodies (IgE) were significantly higher in group inoculated with *Aspergillus niger* spore protein at first sensitization. Research showed that IgE are usually found higher in subjects with symptomatic allergy compared with patients with asymptomatic allergy. The role of immunoglobulin E (IgE) in allergic asthmatic disease is well established (Owen, 2007)

There were structural abnormalities induced by the *Aspergillus niger* spore allergen in the kidney, such as cellular damages, degeneration of the glomeruli and damages of the basement membrane of the Bowman's capsule and collecting tubules (Fig. 2). Inflammatory cells were also recruited in the liver (Fig. 3). The presence of inflammatory cells and associated changes in the liver and kidney could interfer with their functionality and overall health of the animals. In conformity with our findings, Yan *et al.*, xxx showed a relationship between a long term history of allergic disease (allergic asthma and eczema) over 20 years and an acute attack of eczema and asthma before kidney function decreasing. Hafez *et al*, 2009, also showed that Idiopathic nephrotic syndrome, a glomerular group of disease can be precipitated by allergic reactions and has been associated with both aeroallergens (pollens, mold, and dust) and food allergies (Hafez *et al*,2009).

Aspergillus niger is therefore a virulent allergic and pathogenic fungi and being one of the most dominant fungi in both indoors and outdoors environment, is suspected to be among the major allergen triggers that contributes to allergic diseases mobidity, especially in the tropics where environmental conditions favour their growth and sporulation. Though factors such as genetic predisposition, intensity, duration of exposure to allergen and allergen load are among major predisposing factors to their allergens (Lacey and Crook,1988).

Table 1: Differencial immune cells and IgE (Immunoglobulin E) recorded in mice at 1st sensitization

Immune cells	Group 1	Control
Lymphocyte	$50.33+ 0.57^{a}$	$76.0+4.16^{b}$
Neutrophil	$40.33+0.58^{a}$	$16.6+0.58^{b}$
Ring neutrophil	$9.67+0.58^{a}$	$2.00+0.00^{b}$
Monocyte	$14.00+0.00^{a}$	$2.00+0.00^{b}$
IgE	$12.00+0.00^{a}$	$11.93+1.6^{b}$

Values are mean±SD, values with different letters horizontally are significantly different (P<0.05) whereas values with the same letter horizontally are not significantly different (P<0.05)

Table 2: Differencial immune cells and IgE (Immunoglobulin E) recorded in mice at 4th week of sensitization

Immune cells	Group 1	Control
Lymphocyte	$18.00+7.2^{a}$	$19.00+1.00^{a}$
Neutrophil	$73.33+2.89^{a}$	$81.33+1.15^{a}$
Ring neutrophil	$30.67+1.15^{a}$	$0.00+00^{b}$
Monocyte	$3.33+0.57^{a}$	$2.00+0.00^{b}$
IgE	$13.03+0.58^{a}$	$13.90+0.69^{a}$

Values are mean±SD, values with different letters horizontally are significantly different (P<0.05) whereas values with the same letter horizontally are not significantly different (P<0.05)

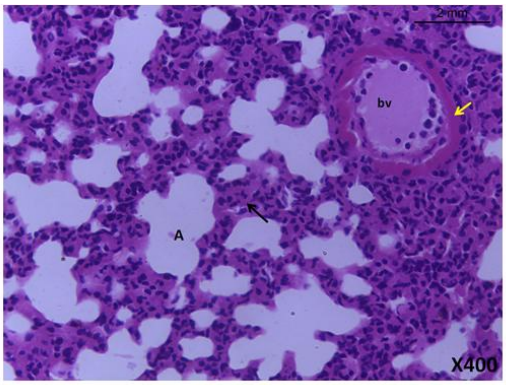

Fig. 1a (lung histology of group 1): **Traces of small darkly inflammatory cell were observed within the blood vessel. (H&E) (bc: bronchioles, bv: blood vessels, black arrow: interalveolar septa, AS: alveolar sac, A: alveoli, Yellow arrow: smooth muscle)**

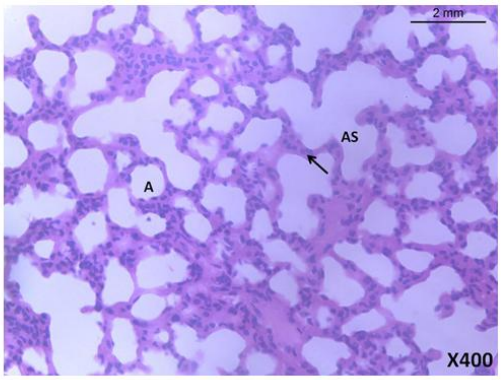

Fig. 1b (lung histology of control): Histology of the lungs showed areas with patchy fibrosis and areas with normal architecture. This might be due to recovery process of the lungs cellular structure (H&E) (bc: bronchioles, bv: blood vessels, black arrow: interalveolar septa, AS: alveolar sac, A:alveoli, Yellow arrow: smooth muscle, blue arrow: columnar shaped cells, asterisk: inflammatory cells, E: edema)

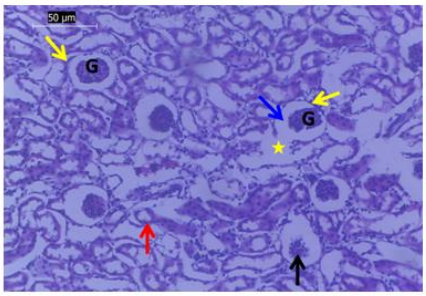

Fig. 2a (kidney histology of group1); Photomicrograph of the kidney showed evidences of cellular damages,, Degeneration of the glomeruli and damages of the basement membrane of the Bowman's capsule and collecting tubules were observed (Yellow arrow: Bowman's capsule, blue arrow: urinary space, G: glomeruli, black arrow: atrophied glomeruli, yellow star: damaged basement membrane, red arrow: PCT)

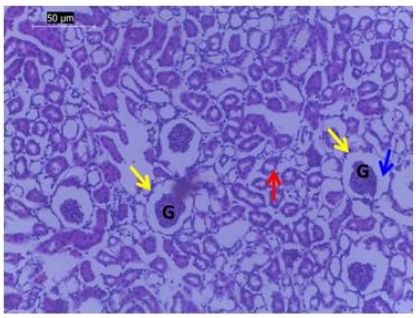

Fig 2b (kidney histology of control group) Photomicrograph of the kidney appeared preserved. (Yellow arrow: Bowman's capsule, blue arrow: urinary space, G: glomeruli, black arrow: atrophied glomeruli, red arrow: PCT)

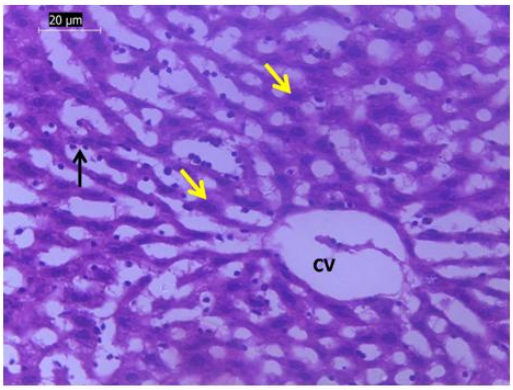

Fig. 3a(liver histology of group 1); Photomicrograph of the hepatic tissue of group 1, at higher magnification showed mild infiltration of inflammatory cells within the parenchyma particularly in the sinusoid of the hepatic tissue. The vascular vessels appeared uncongested. (CV: central vein, yellow arrow: hepatocytes, inflammatory cell

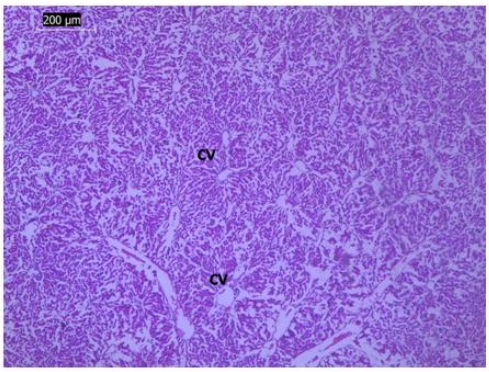

Fig. 3b (liver histology of control:Photomicrograph of the hepatic tissue of C14 B showed moderate alterations cyto-architecture of the hepatic tissue (CV: central vein)

IV. Conclusion

Aspergillus niger spores are allergenic and have the propensity to cause pathologies in the lungs and other vital organs such as liver and kidney. Asthmatics and other hypersensitive individuals should enhance adaptive strategies towards reducing exposures to their spores which are preponderant in indoors and outdoors environment.

V. Recommendation

Adaptive strategies against *Aspergillus niger* spores include but not limited to reducing wetness and increasing ventilation in indoors. Avoiding dust laden in carpets, rugs etc, regular deposal of waste bin, as they are always components of organic waste and release enormous spores as degradation progresses.

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