Journal of Infection (2005) **51**, 375–382

www.elsevierhealth.com/journals/jinf

Interaction of airway epithelial cells (A549) with spores and mycelium of *Aspergillus fumigatus*

Zhihong Zhang[a,b], Rongyu Liu[a,b,*], Jacobien A. Noordhoek[c], Henk F. Kauffman[c]

[a]*Department of Pulmonology, The First Affiliated Hospital of Anhui Medical University, Jixi Road 218, Hefei, Anhui 230022, China*
[b]*Department of Geriatric Institute of Anhui, Hefei, Anhui 230022, China*
[c]*Department of Allergology and Pulmonology, Groningen University Hospital, 9713 GZ Groningen, The Netherlands*

Accepted 23 December 2004
Available online 29 January 2005

KEYWORDS
A. fumigatus;
Airway epithelial cell line;
Interleukin;
Toll-like receptor;
Spore;
Mycelium fragment;
Zymosan

Abstract *Objective*. To study the interaction of airway epithelial cell line A549 with fragments of mycelium, spores of *Aspergillus fumigatus* in vitro and to determine if toll-like receptors (TLRs) are involved in the process.

Methods. A549 cells were exposed to fragments of *A. fumigatus* mycelium, zymosan and inactivated *A. fumigatus* spores. Interleukin 6 (IL-6) and IL-8 released by A549 cells to the culture supernatant were measured by ELISA. Presence of TLR2 and TLR4 on A549 cells were studied by immuno-histochemisty.

Results. Mycelium fragments of *A. fumigatus* showed strong binding to epithelial cells but had limited effects on the release of IL-6 and IL-8 by A549 cells. Irradiated *A. fumigatus* spores were partly internalised by A549 cells and inhibited A549 cells to produce IL-6. TNF-α pre-incubated A549 cells produced increased IL-6 after exposure to zymosan and WIAF. Immuno-histochemistry showed a negative staining for TLR2 and TLR4.

Conclusions. The low levels of cytokines produced by A549 cells after the firm binding of either mycelium or spores of *A. fumigatus* may lead to insufficient recruitment of inflammatory cells to the infected site, which may result in the escape of detection by the immune defence system. TLR2 and TLR4 are probably not or only in part involved in the above process, although very low expression cannot be excluded.

Introduction

Aspergillus fumigatus is a ubiquitous fungus with airborne conidia with a diameter of 2–3 μm, small enough to penetrate deeply into the airways and may reach the lung alveoli. Inhalation of conidia

Abbreviations HAF, heated fragments of mycelium; UAF, mycelium fragments not-treated; WIAF, irradiated and washed fragments; UWIAF, non-washed WIAF fragments.

* Corresponding author. Tel.: +86 551 2922342; fax: +86 551 5120742.
E-mail address: anhuigi@mail.hf.ah.cn (R. Liu).

0163-4453/$30.00
doi:10.1016/j.jinf.2004.12.012

and adhesion to epithelial cells generally is followed by germination and formation of hyphae which is the major characteristic of invasive manifestation of aspergillosis.[1] In patients with allergic bronchopulmonary aspergillosis, it has been shown that *Aspergillus* hyphae grow on and between epithelial cells suggesting that hyphal growth is dependent on the binding of spores to cell surface structures.[2] Furthermore, sensitization to fungal antigens has been associated with more severe and life-threatening asthmatic reactions, especially in childhood asthma.[3,4] The epithelium of the airways plays a key position to participate in local airway inflammation, which may thus contribute to the pathogenesis of airway disease.[5] Recognition systems employed by airway epithelial cells to respond to microbial exposure may include the action of the toll-like receptors (TLRs), which are pattern recognition receptors that recognize conserved molecular patterns on microbes[6,7] and link innate and adaptive immune systems.[8]

The production of cytokines by the airway epithelium such as IL-6 and IL-8 activated by environmental allergens may play a role in causing inflammation associated with respiratory diseases.[9] IL-6 can stimulate the mucin genes, which results in mucous secretion in airways.[10,11] In addition, IL-6 enhances the life-span (survival) of different cell types.[12] In transgenic animal models, overexpression of IL-6 results in sub-epithelial fibrosis.[13,14] Moreover, IL-6 is one of the cytokines that promotes a Th2-type immune deviation,[15] which induces and regulates IgE production and eosinophil airway infiltration, which is the key to asthma.[16] Recent studies have shown that neutrophils play important roles in acute severe asthma attack and IL-8 is an important mediator of this neutrophila.[17,18] Further more, higher sputum IL-8 was associated with greater declines in FEV1.[19]

The mechanism behind fungal induced asthmatic reactions, especially in aspergillus asthma and bronchopulmonary aspergillosis, is still not well understood. One of the questions related with the initiation phase of the asthmatic exacerbations is the role of spores, airborne mycelium particles and soluble allergenic materials from the cell sap in the induction of the asthmatic reaction. Diffusible products from the spores of *A. fumigatus* can inhibit normal alveolar macrophage to produce some cytokines at transcriptional level,[20] suggesting that inhalation of spores may impair the responsiveness of airway cells. However, fungal extracts and fungal proteases can activate cultured epithelial cells to produce cytokines and/or induce epithelial cell detachment.[21,22] The first contact of spores or mycelium of *A. fumigatus* with airway epithelial cells is of great importance.[23] In previous studies from the laboratory in Groningen, A549 cells showed similar characteristics in release of cytokines compared to primary cells from the human airways after challenged with culture-derived allergens from *A. fumigatus*.[21,22] For reasons of comparison with our previous work, we continued the studies with the A549 cell line. In this study, we investigated the activation of cultured airway epithelial cells (A549) with fragments of mycelium and spores of *A. fumigatus*. In addition zymosan was used as a standard model for yeast-capsule induced activation. As parameters of activation we used the release of the proinflammatory cytokine IL-6 and chemokine IL-8 by airway epithelial cells, and which may play a role in the inflammatory response in asthma. Since, toll-like receptors have shown to be of importance in the innate recognition by monocytes[24,25] we were also interested if TLRs are involved in the innate recognition by airway epithelial cells.

Material and methods

Stimuli

Zymosan A from Saccharomyces cerevisiae (Sigma-Aldrich, Zwijndrecht, The Netherlands). A stock solution of 1000 μg/ml in RPMI 1640 was prepared and stored at −80 °C.

A. fumigatus was a pure culture isolated from the sputum of a patient with an aspergilloma from the Groningen University Hospital. Cultured in Sabouraud medium for 5 days the mycelium mass of *A. fumigatus* was harvested. The mycelium fraction was washed with PBS and dried by pressing with filter paper and stored at −80 °C. After thawing, the mycelium was resuspended in PBS and mechanically destructed (30 min on ice) and frozen again. This procedure was repeated three times. Finally, the fractionated mycelium fragments were centrifuged for 10 min at 1880 g. The short fragments of mycelium obtained in this way contained only 3–8 cells in each fragment. The density of mycelium fragments was determined using a Bürker Türk chamber. A stock solution of 8.0×10^6 fragments/ml PBS of untreated fragments (UAF) was prepared and stored at −80 °C.

Since, some cells in the fragments were still viable we also prepared heated fragments of *A. fumigatus* (HAF) by heating them in a water bath at 90 °C for 1 h (stock solution 8.0×10^6 fragments/ml PBS). Additionally, we prepared irradiated fragments of *A. fumigatus* mycelium by exposing them