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The Effects of Different Fungi on the IL-1β Expression in Mouse Dendritic Cells

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Abstract

Background: Invasive fungal infection received more and more attention because of its high mortality, *Candida albicans* and *Aspergillus fumigatus* is the most common pathogenic fungus for systematic fungal infection, *A. lentulus* was isolated and identified recently and named as a sister of *A. fumigatus*.

Objectives: The current study aimed to explore the concentration and time-dependent relationships of the virulence of fungi due to the change in the interleukin-1 beta (IL-1β) level.

Methods: *Candida albicans*, *A. fumigatus*, and *A. lentulus* suspensions with a multiplicity of infection = 0, 1, 5, 10, and 20 units were used to treat mouse dendritic cells. The IL-β level was measured by enzyme-linked immunosorbent assay (ELISA) at 2, 6, 12, 24, and 48 hours after the treatment was administered.

Results: The main effects and interactions between the multiplicity of infection, intervention duration, and the dependent variable of IL-1β were significant. Besides, there were statistically significant differences. Only *C. albicans* and *A. lentulus* could induce IL-1β 48 hours after administration. Furthermore, the production of IL-1β induced by *A. fumigatus* was higher than that induced by *A. lentulus* and *C. albicans*.

Conclusions: This study demonstrated concentration- and time-dependent relationships in IL-1β production by dendritic cells induced by three types of fungi. *Candida albicans* and *A. lentulus* exhibited a slow phase-in *in vitro* inflammation induction. The inflammatory response induced by *A. fumigatus in vitro* has the characteristics of a short action time and a strong toxic effect. Finally, *A. lentulus* is less virulent than *A. fumigatus*, and its inflammation-inducing time is relatively longer.

Keywords: *Candida albicans*, *Aspergillus fumigatus*, *A. lentulus*, IL-1β

1. Background

The incidence of opportunistic fungal infections is on the rise, mainly due to population aging and enhanced incidence of malignant tumors. *Candida* is the most common cause of digestive tract infection, while *Aspergillus* is known as the most likely cause of invasive lung infection (1). In China, the mortality of untreated pulmonary *Aspergillus* infection is high (2-4), and *Aspergillus fumigatus* infections account for 70 - 80% of pulmonary *Aspergillus* infections (5). Recent developments in molecular-based approaches have resulted in detecting new species of *Aspergillus* (6). These rare *Aspergillus* are different from *A. fumigatus* in toxicology, drug resistance, and growth conditions (6-8). *Aspergillus lentulus* was first isolated and identified in patients with leukemia by Balajee in 2005 and named as a sister of *A. fumigatus* (9).

Subsequently, scholars could isolate *A. lentulus* from bronchoalveolar lavage fluid and sputum samples of patients who underwent heart, liver, and kidney transplantations and patients with chronic obstructive pulmonary emphysema and cystic fibrosis in Japan (10), Brazil (11), Switzerland (12), Spain (13), Argentina (14), Turkey (15), and other countries. Therefore, it has attracted more attention over the years. In 2011, our team isolated *A. lentulus* from the sputum of an elderly male patient who had chronic pulmonary heart disease and acute pulmonary infection for 5 years (16). Finally, the patient died of invasive pulmonary fungal infection despite receiving active treatment. This was the first time that Chinese scholars could find cases of *A. lentulus* infection and cases of non-immune deficiency infection in China. However, the virulence and host im-

mune response pathway of the fungus remain unclear.

Natural immunity is the most important line of defense against fungal infections, and the NACHT, LRR, and PYD domains-containing protein 3 (NLRP3) inflammatory corpuscle pathway plays a crucial in fungus-induced host immune response. Fungi can induce the NLRP3 inflammatory pathway, which in turn causes releasing inflammatory factors by infected cells, such as tumor necrosis factor-α (TNF-α) and interleukin-1 beta (IL-1β), to resist against fungal invasion. IL-1β is an important immunomodulator against infection and injury in the NLRP3 pathway. It has also been reported that IL-1β is required for the host to inhibit *Candida albicans* infection (16-19).

2. Objectives

The current study aimed to investigate the ability of three fungi (i.e. *C. albicans*, *A. fumigatus*, and *A. lentulus*) to induce the host immune response of mouse dendritic cells by detecting the production of IL-1β.

3. Methods

The experimental cells that included DC2.4 mouse dendritic cells, a cell type, were cultured in RPMI-1640 medium + 10% fetal bovine serum + 1% phosphate-buffered saline (PBS) with 5% CO2 at 37°C and saturated humidity (5). The experimental fungi were *C. albicans*, *A. fumigatus*, and *A. lentulus*.

3.1. The Cell Cultures and Fungal Cultures

The frozen cells were taken out of the liquid nitrogen and immediately placed in a 37°C water bath, and the cryopreservation tube was shaken quickly. Then, the cells were thawed quickly for two minutes. Furthermore, 9 mL of complete medium was added to a 15 mL centrifuge tube in advance. The thawed cells were quickly added to the 15 mL centrifuge tube and then centrifuged, and the supernatant was discarded. The cells were transferred to a culture flask, cultured at 37°C with saturated humidity and 5% CO_2 in an incubator, and sub-cultured for future use. The DC2.4 cells, with a good confluence rate of 90%, were taken, trypsinized, and passaged at a ratio of 1:4 to the 7th day.

Lipopolysaccharide with a final concentration of 1 mg/L was added for 48 hours. Afterward, the suspended cells were collected and passaged at a ratio of 1:3, and the cell suspension was collected before the experiment. Using Sabouraud medium, *C. albicans* and *A. fumigatus* were cultured at 37°C for 4 days, and *A. lentulus* was cultured at 37°C for 9 days (6). The selected boll glucose agar was produced by Becton, Dickinson and Company (New York, NJ, USA, date of production: 2017-02, a period of validity: 2022-01, batch number of production: 7109972). When used, 19.5 g of prepared medium powder was dissolved in 500 mL of distilled water. After full dissolution, the pressure cooker was disinfected and sterilized. The powder was placed in a MEA 9 cm petri dish on the sterile operating table and stored at low temperature.

The cell concentration was adjusted to 5×10^4 cells/mL with complete medium (10% serum RPMI-1640 complete medium: 10 mL of fetal bovine serum was added to 90 mL of RPMI-1640 medium, and 1 mL of a dual-antibody solution of penicillin-streptomycin was added. The concentration of the cell suspension was adjusted to 5×10^4 cells/mL using complete medium) and the cells were inoculated into a 24-well plate, 500 uL/well. The cultured *C. albicans*, *A. fumigatus*, and *A. lentulus* were grinded and then rinsed with sterile PBS three times, counted using a counting board, and then the DC2.4 cells were infected with these fungi with a multiplicity of infection = 0, 1, 5, 10, and 20 units. After 2, 6, 12, 24, and 48 hours of infection, the supernatant was collected.

3.2. The Detection of IL-1β

In strict accordance with the instructions of the mouse IL-1β enzyme-linked immunosorbent assay (ELISA) kit (MULTI SCIENCES, Hangzhou, China), the supernatant of the cell culture medium of the above groups was detected by ELISA.

3.3. Statistical Analysis

Data were analyzed using SPSS version 19.0 (International Business Machines Corporation, New York, America). Data are expressed as mean $\pm$ standard deviation (X $\pm$ SD). Initially, multivariate analysis of variance (ANOVA) was used to analyze the correlation between the main effect, the interaction effect, and the dependent variables. Then, univariate ANOVA was performed. If the data were normally distributed, univariate ANOVA was used. Data were analyzed by multiple comparisons using the least significant difference method, and data with heterogeneity of variance underwent multiple comparisons using the Tamhane method. Statistical significance was considered when P-value < 0.05. For skewed data, log transformation was used to approximately conform to normality. Then, the ANOVA method was used to analyze the data.

4. Results

4.1. The Analysis of the Relationship Between the Production of IL-1β Induced by Three Types of Fungi and Their Action Time and Concentration

The main effects and interactions among the multiplicity of infection value, intervention duration, and the dependent variable IL-1β among *C. albicans*, *A. fumigatus*, and *A. lentulus* were significant, and the differences were statistically significant (Tables 1-3). Based on the findings, action time and multiplicity of infection value contributed to the increase in IL-1β induced by the three types of fungi. Besides, we found an interaction between them.

4.2. The Two-Factor Analysis of IL-1β Production by Three Types of Fungi with Different Multiplicity of Infection Values at Different Intervention Times

As mentioned before, the IL-1β production induced by the three types of fungi was related to the action time and concentration. Thereafter, a two-factor analysis of IL-1β production by three types of fungi with different multiplicity of infection values at different intervention times was performed (Figures 1 and 2). The increase in IL-1β could only be induced by *C. albicans* after 48 hours of treatment and by *A. fumigatus* after 12 hours of treatment; the highest level of toxicity was observed after 48 hours, and multiplicity of infection was one unit. Similar to *C. albicans*, the increase in IL-1β could only be induced by *A. lentulus* after 48 hours of treatment; however, in contrast to *C. albicans*, it induced a significant increase when multiplicity of infection was one, that is, the inflammatory effect of *A. lentulus* was stronger than that of *C. albicans*.

4.3. The Analysis of the Difference in IL-1β Among the Three Groups at the Same Time and the Same Multiplicity of Infection

Finally, to determine the magnitude of the inflammation-inducing effect of the three types of fungi, the difference in IL-1β level among the three groups at the same time and the same multiplicity of infection was analyzed. After treating the three groups with the mouse dendritic cells at 12 hours, the IL-1β level was increased significantly, and at 12, 24, and 48 hours, the IL-1β level was higher in the *A. fumigatus* treated group than the *C. albicans* and *A. lentulus* groups, and the differences were statistically significant. The highest level of IL-1β was found at 48 hours in all three groups; however, the best multiplicity of infection value was different: multiplicity of infection = 20 in *C. albicans*, multiplicity of infection = 1 in *A. fumigatus*, and multiplicity of infection = 5 in *A. lentulus*. At the same time and with similar value of multiplicity of infection, the inflammation-inducing effect of *A. fumigatus* was always greater than that of *A. lentulus*. In the *C. albicans* group, the inflammation-inducing effect was continuously and positively correlated with time and concentration.

5. Discussion

Candida is the most common cause of digestive tract infection, and *Aspergillus* is an invasive lung infection caused by *Aspergillus* (1). Moreover, in recent years, new species of *Aspergillus* are reported; however, their toxicology, drug resistance, and growth conditions have not been fully defined. *A. lentulus* is a new *Aspergillus* that was identified in 2005 by Balajee and gradually attracted researchers' attention (11). However, still the virulence and host immune response pathway of this fungus are unclear. To a certain extent, virulence contributes to the inflammatory response induced by fungus. In this study, the level of IL-β after the action of *C. albicans*, *A. fumigatus*, and *A. lentulus* on the mouse dendritic cells was measured to indirectly reflect the inflammation-inducing effect and virulence of the three types of fungi.

This study demonstrated that the production of IL-1β induced by the three fungi depended on their concentration and action duration, where the inflammation-inducing effect of *C. albicans* presented significant concentration and time-dependent patterns. Its virulence is weaker than that of *A. fumigatus*, because *C. albicans* can stimulate the NLRP3 activation only when it changes from the yeast phase to the mycelium phase (19-22). A previous study on the host immune response of *A. fumigatus* revealed that the IL-1β level was significantly increased in human dendritic cells 6 hours after being treated with the fragment of *A. fumigatus*; therefore, *A. fumigatus* had the characteristic of fast inflammation (23).

This finding is consistent with the finding of our study. *Aspergillus lentulus* is a new strain, and sufficient evidence about its virulence and host immune response are not available. It's well-documented that *A. lentulus* can infect not only immunocompromised patients but also patients with non-granulocytopenia, and the disease has a very high mortality rate. The present study revealed that the inflammation-inducing effect of *A. lentulus* was weaker than that of *A. fumigatus* at the same concentration and time, and its high mortality rate can be attributed to its

Table 1. Intersubjective Effect for *Candida albicans*[a]

Items	III-Type Quadratic Sum	df	Mean Square	F	Sig.
Correction model	2271.938[a]	24	94.664	45.226	0.000
Nodal increment	8306.677	1	8306.677	3968.494	0.000
Multiplicity of infection	449.367	4	112.342	53.671	0.000
Intervention durations	410.549	4	102.637	49.035	0.000
Multiplicity of infection * intervention durations	1412.022	16	88.251	42.162	0.000
Error	104.658	50	2.093		
Total	10683.273	75			
Corrected total	2376.596	74			

[a] R square = 0.956 (adjusted R square = 0.935).

Table 2. Intersubjective Effect for *Aspergillus fumigatus*

Items	III-Type Quadratic Sum	df	Mean Square	F	Sig.
Correction model	17314.092[a]	24	721.421	261.855	0.000
Nodal increment	22496.117	1	22496.117	8165.453	0.000
Multiplicity of infection	1444.550	4	361.138	131.083	0.000
Intervention durations	9927.632	4	2481.908	900.862	0.000
Multiplicity of infection * intervention durations	5941.910	16	371.369	134.797	0.000
Error	137.752	50	2.755		
Total	39947.961	75			
Corrected total	17451.844	74			

[a] R square = 0.992 (adjusted R square = 0.988).

Table 3. Intersubjective Effect for *Aspergillus lentulus*

Items	III-Type Quadratic Sum	df	Mean Square	F	Sig.
Correction model	1756.644[a]	24	73.194	57.040	0.000
Nodal increment	8374.738	1	8374.738	6526.471	0.000
Multiplicity of infection	194.161	4	48.540	37.828	0.000
Intervention durations	959.601	4	239.900	186.955	0.000
Multiplicity of infection * intervention durations	602.882	16	37.680	29.364	0.000
Error	64.160	50	1.283		
Total	10195.542	75			
Corrected total	1820.804	74			

[a] R square = 0.965 (adjusted R square = 0.948).

high resistance to currently available drugs. This is consistent with the results of previous studies and our previous drug sensitivity tests (23).

Some scholars have used the larvae infection model to investigate this issue, and reported that *A. fumigatus* was more virulent than *A. lentulus*. In these previously conducted studies, two days after the infection, the active mycelial growth of *A. fumigatus* was not visible in the lar-

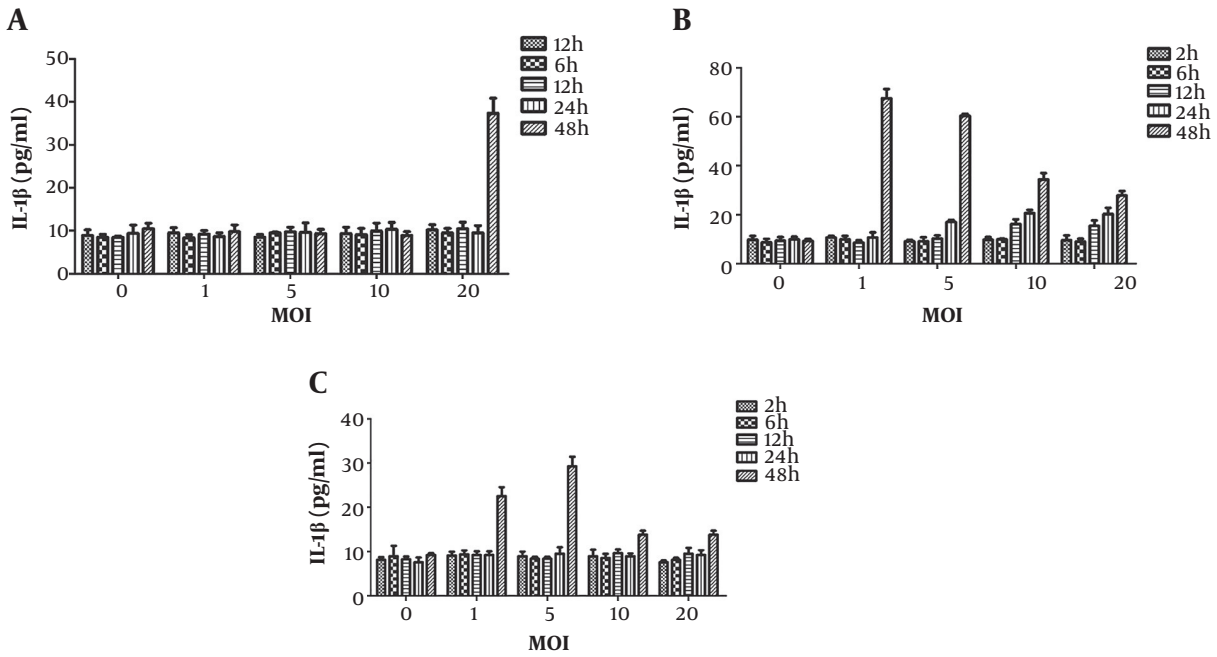

Figure 1. The effect of different fungi with different intervention durations on the level of IL-1β in the supernatant of DC mouse dendritic cells. A, The effect of *Candida* with different multiplicity of infection values and different intervention durations on the level of IL-1β in the supernatant of DC mouse dendritic cells; B, the effect of *Aspergillus fumigatus* with different multiplicity of infection values and different intervention durations on the level of IL-1β in the supernatant of DC mouse dendritic cells; C, the effects of *A. lentulus* with different multiplicity of infection values and different intervention durations on the level of IL-1β in the supernatant of DC mouse dendritic cells. MOI, multiplicity of infection; DC, dendritic cells.

vae tissue section, whereas *A. lentulus* grew slowly and was surrounded by the adjacent tissue cells (20). In this *in vitro* study, compared to *A. fumigatus*, *A. lentulus* showed a shorter duration of inflammation, which further supports the fact that *A. lentulus* belongs to slow phase fungi and its multidrug resistance may be an important factor for its poor prognosis. However, further studies are needed to prove this hypothesis. Moreover, further studies on different cells and animal models are needed to investigate relevant mechanisms.

5.1. Conclusions

There are concentration and time-dependent relationships in IL-1β production by dendritic cells induced by *A. fumigatus*, *C. albicans*, and *A. lentulus*. Besides, *A. lentulus* exhibited a slow phase in *in vitro* inflammation induction. The inflammatory response induced by *A. fumigatus in vitro* has the characteristics of a short action time and a strong toxic effect. Finally, *A. lentulus* is less virulent than *A. fumigatus* and the inflammation-inducing time is relatively longer.

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Footnotes

Authors' Contribution: Li-Juan Zhang and Paride Abliz did substantial contributions to the conception and design of the work, and draft the work. Li-Juan Zhang, Xiao-Dong Wang, Xue-Feng Wan, Hadiliya Hasimu, and Paride Abliz did the acquisition, analysis, and interpretation of data for the work. Li-Juan Zhang, Xiao-Dong Wang, Xue-Feng Wan, Hadiliya Hasimu, and Paride Abliz did revising it critically for important intellectual content. Li-Juan Zhang, Xiao-Dong Wang, Xue-Feng Wan, Hadiliya Hasimu, and Paride Abliz did final approval of the version to be published. Li-Juan Zhang, Xiao-Dong Wang, Xue-Feng Wan, Hadiliya Hasimu, and Paride Abliz did agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

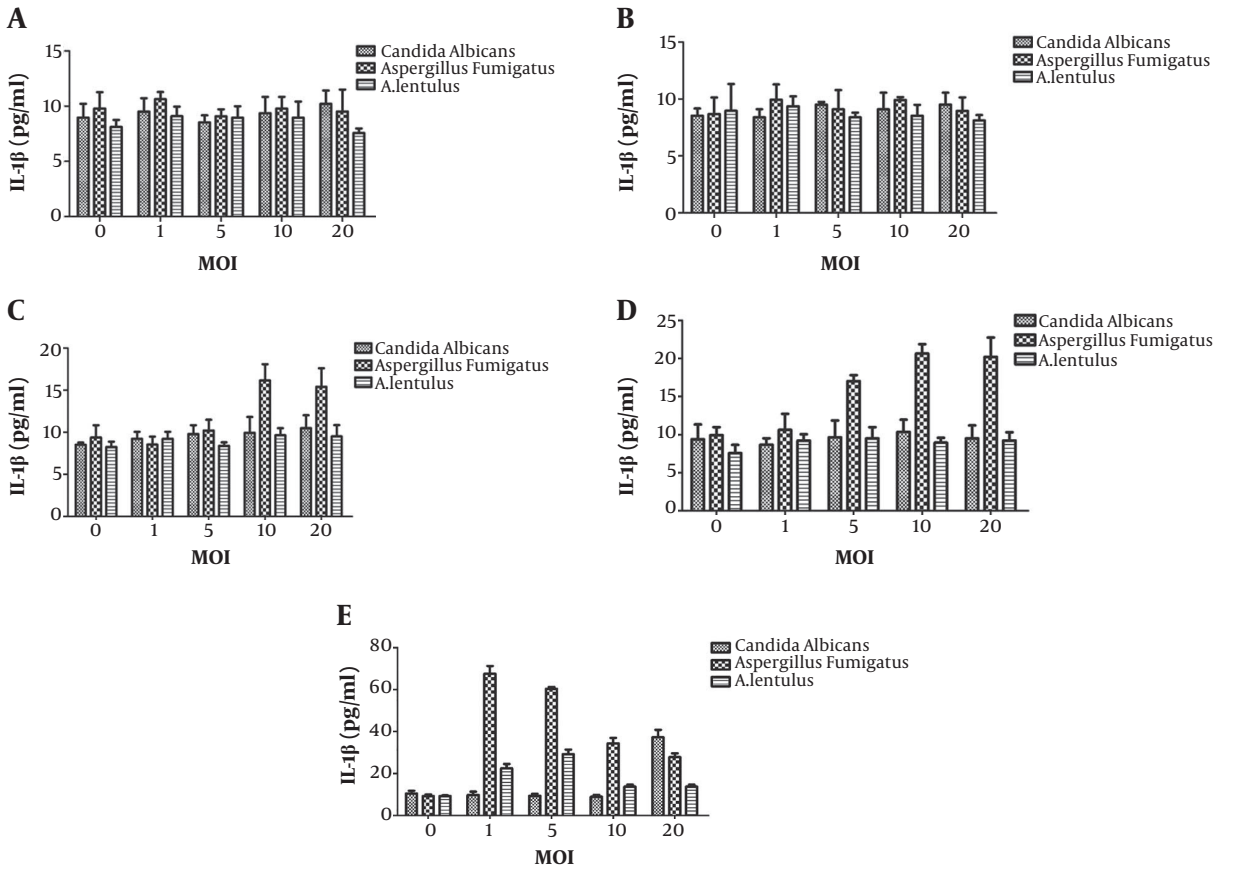

Figure 2. The effect of different fungi with different intervention durations on the level of IL-1β in the supernatant of DC mouse dendritic cells. A, The effect of different fungi with 2 hours of intervention on the level of IL-1β in the supernatant of DC mouse dendritic cells; B, the effect of different fungi with 6 hours of intervention on the level of IL-1β in the supernatant of DC mouse dendritic cells; C, the effect of different fungi with 12 hours of intervention on the level of IL-1β in the supernatant of DC mouse dendritic cells; D, the effect of different fungi with 24 hours of intervention on the level of IL-1β in the supernatant of DC mouse dendritic cells; E, the effect of different fungi with 48 hours of intervention on the level of IL-1β in the supernatant of DC mouse dendritic cells. MOI, multiplicity of infection; DC, dendritic cells.

Conflict of Interests: The authors declare no conflict of interest.

Ethical Approval: This study is approved by the Ethics Committee of The First Affiliated Hospital of Xinjiang Medical University.

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