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Dectin-2 is predominately macrophage restricted and exhibits conspicuous expression during *Aspergillus fumigatus* invasion in human lung

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ABSTRACT

We investigated the features of Dectin-2 expression both at transcriptional and translational levels during *Aspergillus fumigatus* infection in human lung. Simultaneously, the expression of CD206 was assayed as an activated marker of alveolar macrophages. The characteristic of Dectin-2 expression were then confirmed in Monocyte-derived macrophages (MDM) after *A. fumigatus* stimulation by Flow Cytometry. We found that the expression of Dectin-2 was low in normal lung, while it revealed a markedly up-regulation during *A. fumigatus* invasion. Dectin-2 expression was predominantly restricted to CD206 positive cells. There was salient positive correlation between Dectin-2 expression and CD206. We conclude that Dectin-2 expression is largely restricted to alveolar macrophages in human lung. The conspicuous expression of Dectin-2 during *A. fumigatus* invasion suggests its notable contribution to antifungal defenses in pulmonary aspergillosis.

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1. Introduction

Aspergillosis is the most common mold infection worldwide, and *Aspergillus fumigatus* accounts for over 90% of all reported cases of this disease [1,2]. Due to the daily inhalation and abundance of *A. fumigatus* spores in surroundings, respiratory system is the leading susceptible target of this pathogen, and the infection on respiratory system accounts for an overwhelming majority of the reported cases [2,3]. The diagnosis and treatment for this disease are usually very difficult because of the wide spectrum of the disease, and the different susceptibility to *A. fumigatus* invasion and thus the different impairment of host immunity [2,4], which makes the outcome of this disease often fatal. Therefore, further detection of the immune mechanisms induced by *A. fumigatus* invasion is vital for better diagnosis and prognosis of the diseases.

Monocytes/macrophages and dendritic cells are the sentinel of innate immunity. Through recognition by pattern recognition receptors (PRRs) of pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) present during fungal infections, these antigen presenting cells (APC) play essential roles in host defense against fungal infections [5–8]. C-type lectins receptors (CLRS) are important members in PRRs. Through recognizing mainly the carbohydrate structures, CLRS play pivotal roles in activating innate immunity and host defense against fungi [5,9,10]. Dectin-2, also known as CLEC6A, is a recently detected CLR that is primarily expressed in dendritic cells and macrophages. Dectin-2 plays an important role in the activation of various genes, including those encoding pro-inflammatory cytokines through recognizing high mannose-type carbohydrates on various fungal cells [11,12]. By activating the vital downstream signaling kinase Syk, Dectin-2 is documented to mediate respiratory burst as well as producing cytokines that preferentially induce the differentiation of Th17 cells *in vitro* [13,14]. Furthermore, Dectin-2-deficient mice showed decreased survival during the infection of different strains of *Candida albicans* [11,12,15]. Although the high mannose-type carbohydrates exist in various fungi, very little is known about the expression of Dectin-2 in human lungs and its role in immune defense against *Aspergillus* invasion.

The human Macrophage Mannose Receptor (MMR), also known as CD206, is another member of the CLR family primarily expressed in tissue macrophages and myeloid dendritic cells. Macrophages can be polarized into classically activated macrophages (M1) and alternatively activated macrophages (M2) in response to the microenvironment, resulting in phenotype and physiology changes. Previous studies have shown that CD206 is not expressed in M1 macrophages and therefore serves as a useful marker for M2

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macrophages [16–19]. Considering that Dectin-2 and CD206 coexist in tissue macrophages and both recognize high mannose-type carbohydrates in fungi, in this study we chose CD206 as a marker of macrophages to identify the cell specific expression of Dectin-2, and then further analyzed the characteristic expression of Dectin-2 in different pathophysiological states during *A. fumigatus* invasion. Our results indicate that Dectin-2 plays an essential role in innate immunity and has notable contribution to antifungal defenses in pulmonary aspergillosis.

2. Materials and methods

2.1. Ethical permission

The study protocol and consent forms conform to the Declaration of Helsinki and were approved by the Ethical Review Board (ERB) Committee (Jinling Hospital, Nanjing, China). The informed consent was obtained from all subjects.

2.2. Patients and samples

Seven surgically removed *A. fumigatus*-infected and matched normal lung specimens were obtained from patients in Jinling Hospital (from June 2010 to May 2012). Two patients were diagnosed with allergic bronchial pulmonary aspergillosis (ABPA), and five were diagnosed with invasive pulmonary aspergillosis (IPA). Patients who received pre-operative anti-fungal therapy were excluded. All diagnoses conformed to current standard criteria and were confirmed by pathology of the biopsy specimens with detailed information shown in Table 1.

2.3. Reagents and antibodies

The RNAiso Plus and PrimeScript RT Master Mix were purchased from TaKaRa (Dalian, China). The FastStart Universal SYBR Green Master (ROX) was obtained from Roche (Shanghai, China). Polymerase chain reaction (PCR) primers were designed and synthesized by Invitrogen (Shanghai, China). Phosphatase inhibitor cocktail (cat. No. 04906845001) was purchased from Roche (Mannheim, Germany), protease inhibitor cocktail (No. R1321) was purchased from Fermentas UAB (Vilnius, Lithuania). Protein assay reagent and an enhanced chemiluminescent (ECL) kit were purchased from Pierce (Rockford, IL). Histological and immunohistochemistry reagents and ready-to-use secondary antibody kit were purchased from Maixin Biotechnology (Fuzhou, China). RPMI 1640 medium and 10% heat-treated fetal bovine serum (FBS) were purchased from Wisent Inc (Quebec, Austria). 10% human AB serum was obtained from Roche. Antibodies to Dectin-2 (AF3114) and CD206 (MAB25342) were obtained from R&D Systems (MN, USA). Antibodies to β-actin (antibody 4967) and horseradish peroxidase (HRP) were from Cell Signaling Technology (Beverly, MA, USA); FITC-conjugated Rabbit Anti-Goat IgG was obtained from Jackson ImmunoResearch (West Grove, PA, USA). Anti-human CD206 PE was purchased from eBioscience (San Diego, USA).

Table 2
Sequences of primers for quantitative real-time RT-PCR.

Primers	Sequence
Forward Primer (Dectin-2)	5′-GCTTTCAGACCCACAAGGTAAT-3′
Reverse Primer (Dectin-2)	5′-GCAGAATGATTGGGCTCACCTA-3′
Forward Primer (CD206)	5′-AAGGCGGTGACCTCACAAG-3′
Reverse Primer (CD206)	5′-AAAGTCCAATTCCTCGATGGTG-3′
Forward Primer (GADPH)	5′-AAGGTGAAGGTCGGAGTCAAC-3′
Reverse Primer (GADPH)	5′-GGGGTCATTGATGGCAACAATA-3′

2.4. Real time quantitative RT-PCR assays

Total RNAs were extracted from the frozen tissue specimens by using RNAiso Reagent according to the manufacturer's instructions (TaKaRa, Dalian, China). cDNA was synthesized with the First-Strand cDNA Synthesis kit according to the manufacture's protocol (TaKaRa, Dalian, China). The quantitative real-time RT-PCR (qRT-PCR) was performed by FastStart Universal SYBER Green Master on an ABI 7300 Real Time PCR System (Applied Biosystem, Foster City, USA). The primers used are listed in Table 2. Amplification conditions were as follows: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, 60 °C for 45 s. Melting curve analysis was used to confirm the specificity of primers. The results were expressed at a relative mRNA expression level, calculated using the Pfaffl method, with GAPDH serving as the reference gene [20].

2.5. Western blotting

Western blotting was performed according to the procedures described below. Briefly, frozen specimens were homogenized and lysed in RIPA buffer containing 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS, protease inhibitor cocktail, and phosphatase inhibitor cocktail. Proteins were separated by SDS–PAGE, transferred to polyvinylidene fluoride membranes, blocked with 5% bovine serum albumin (BSA), and then hybridized with Dectin-2 or CD206 antibody (diluted 1:500). After incubation with horseradish peroxidase-conjugated anti-donkey and anti-mouse secondary antibodies (both diluted 1:5000) and further washes, the membranes were developed with Amersham ECL Western blotting detection system (GE Healthcare). The protein band density was

Table 1
General characteristics of the patients.

Age(y)	Gender	Lesion location	Diagnosis	CT manifestation	Pathology of biopsy specimens
55	Male	Anterior basilar segment and medial basilar segment of the right lower lobe	IPA	Nodules accompanied with small cavity, halo sign,inflammatory infiltration	*A. fumigatus* hyphae
42	Female	Left, upper lobe	ABPA	Central bronchiectasis with a high density of mucus plugs, tree in bud sign	*A. fumigatus* hyphae
49	Female	Posterior and medial basilar segment of the right lower lobe	IPA	Nodules accompanied with small cavity, inflammatory infiltration	*A. fumigatus* hyphae
66	Female	superior segment of the right lower lobe	ABPA	Central bronchiectasis, tree in bud sign, nodules	*A. fumigatus* hyphae
50	Female	Anterior basilar segment and medial basilar segment of the right lower lobe	IPA	Spherical lesions accompanied with inflammatory infiltration	*A. fumigatus* hyphae
60	Male	Posterior and medial basilar segment of the right lower lobe	IPA	Multiple nodules accompanied with cavities, inflammatory infiltration	*A. fumigatus* hyphae
45	Male	Medial and anterior basilar segment of the right lower lobe	IPA	Multiple nodules accompanied with halo sign, inflammatory infiltration	*A. fumigatus* hyphae

Abbreviations: ABPA: allergic bronchial and pulmonary aspergillosis. IPA: invasive pulmonary aspergillosis. CT: computer tomography.

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