INSTITUT PASTEUR

Microbes and Infection 11 (2009) 661–670

www.elsevier.com/locate/micinf

Original article

Dectin-1 is required for human dendritic cells to initiate immune response to *Candida albicans* through Syk activation

Franck Skrzypek, Elio Cenci, Donatella Pietrella, Anna Rachini, Francesco Bistoni, Anna Vecchiarelli*

Microbiology Section, Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Via del Giochetto, 06126 Perugia, Italy

Received 18 September 2008; accepted 25 March 2009
Available online 7 April 2009

Abstract

Dectin-1 is a pattern recognition receptor found on monocytes and dendritic cells (DC) able to recognize β-1,3 and β-1,6 glucans. It is thought to act via the spleen tyrosine kinase (Syk) to initiate immune response against infectious agents such as *Candida albicans*, one of the leading causes of invasive fungal disease in immunocompromised individuals. This study addresses the importance of this receptor in the context of human DC response to *C. albicans*. Upon blockage of Dectin-1, immature DC are less able than untreated cells to bind, phagocytose, and kill *C. albicans* via oxidative burst. In fact, a consistent decrease in superoxide anion, but not nitric oxide production, was manifested when the Syk pathway was inhibited. *C. albicans*-induced cytokine production via Dectin-1 recognition is mediated by the Syk activation pathway. Indeed, specific Syk inhibition significantly suppressed the production of IL-12, IL-6, and TNF-α. Finally, we observed that Dectin-1 engagement was also involved in DC maturation and subsequent lymphocyte activation. Collectively, these findings identify Dectin-1 as a key receptor influencing critical biological functions of DC in response to *C. albicans* leading to T cells response alteration. These effects are largely, though not completely, mediated by Syk activation.

Keywords: Dectin-1; Dendritic cells; *C. albicans*; Syk; Cytokine; Immune response

1. Introduction

Candida albicans is a fungal pathogen reported to be one of the leading causes of nosocomial bloodstream infections [1]. Though normally a member of the resident microbial population of the alimentary tract and mucocutaneous membranes of the healthy host, *C. albicans* behaves as an opportunistic pathogen when immune defenses are compromised. The incidence of systemic candidiasis in immunocompromised individuals is accompanied by a mortality rate of nearly 30%, despite the development of new therapeutic strategies [1].

The most important virulence factor in the pathogenesis of *Candida* infections is the morphologic transition from yeast cells to filamentous or hyphal forms, which allow fungi to elude the immune system [2,3]. Indeed, the mechanisms underlying host response to the pathogenic hyphal form of *C. albicans* are not as well understood as those regulating response to the yeast form. The latter, in fact, is subject to phagocytic clearance, intracellular killing, fragmentation, and presentation through MHC class II on the surface of antigen presenting cells such as dendritic cells (DC) [3].

DC precursors are bone marrow-originated and are professional antigen capturing cells able to prime naïve T cells and cross-present antigens. Immature DC are phagocytic cells capable of sampling antigens at the site of injury or infection and able to migrate to secondary lymphoid organs, where they can present antigens to T cells [4–6]. Upon activation with pro-inflammatory stimuli, DC acquire surface expression of co-stimulatory molecules (e.g. CD40, CD80, or CD86) and adhesion molecules including LFA-3 and ICAM-1 [7],

* Corresponding author. Tel./fax: +39 075 585 7407.
E-mail address: vecchiar@unipg.it (A. Vecchiarelli).

1286-4579/$ - see front matter © 2009 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.micinf.2009.03.010

allowing DC to establish antigen-specific interaction with T cells. For DC to be able to recognize, uptake, and phagocytose pathogens, surface expression of IgG and IgE receptors as well as carbohydrate receptors appears to be essential [8,9], since the cell wall of fungi consists mainly of carbohydrates like mannoproteins, β-glucans, and chitin. Among the pattern recognition receptors (PRR) involved in this process, Dectin-1 is dedicated to the recognition of β-1,3 glucans [10], which make up to 50% of the fungal cell wall. Named β-glucan receptor in mice, and firstly identified as DC receptor interacting with an endogenous ligand expressed on T cells, Dectin-1 has been subsequently found in other cell types. Indeed, Dectin-1 is strongly expressed in neutrophils, monocytes, macrophages, and to a lesser degree in subsets of T and B cells, as well as eosinophils [11]. As such, it is considered to be the major macrophage PRR for β-glucans [10]. Dectin-1 has been demonstrated to bind the β-glucan-rich particle zymosan, as well as live *Candida* and many other fungal genera, including *Saccharomyces*, *Coccidioides*, *Pneumocystis*, and *Aspergillus* [10,12–14].

Moreover, Dectin-1 was shown to be required for respiratory burst in response to β-glucans [15], as well as cytokine production in cooperation with toll-like receptors (TLR) 2 and 6 [16]. Dectin-1 is a C-type lectin-like transmembrane receptor containing an ITAM-like motif in its cytoplasmic tail, phosphorylated on ligand-binding [10,17], and able to activate a spleen tyrosine kinase (Syk) dependent pathway [17]. Involvement of Syk activates different pathways like the MAP kinase, CARD9, or PLC pathways [18–20]. Dectin-1 has been studied intensively on mouse cells, but very few works have been done so far exploring its role in the interaction between *C. albicans* and human cells, particularly DC.

Here, the contribution of Dectin-1 to the interaction between immature human DC and *C. albicans* has been assessed.

2. Materials and methods

2.1. Reagents and antibodies

Piceatannol was purchased from Calbiochem (La Jolla, CA). MyD88 Homodimerization inhibitory peptide set was purchased from Imgenex (San Diego, CA). Human recombinant granulocyte macrophage colony stimulating factor (GM-CSF), Human recombinant macrophage colony stimulating factor (M-CSF), IL-4 and IFN-γ were purchased from Immuno Tools (Friesoythe, Germany). 4,5-diaminofluorescein (DAF-2) was purchased from Cayman Chemical (Ann Arbor, MI). 2′,7′-dichlorodihydrofluorescein diacetate (DCDHF) was purchased from Alexis Biochemicals (Lausen, Switzerland). Goat polyclonal antibody against the human receptor Dectin-1 (Dec1Ab) was purchased from R&D Systems (Minneapolis, MN). Goat IgG irrelevant control antibody (IrrAb) was purchased from Bethyl laboratories (Montgomery, TX). Laminarin was obtained from Sigma.

2.2. Monocyte isolation, dendritic cells and monocyte-derived macrophages generation

DC and monocyte-derived macrophages (MDM) were generated from human peripheral blood monocytes (PBM) from heparinized venous blood obtained from healthy donors as previously described [21]. Blood was diluted with RPMI 1640 (Gibco, Paisley, Scotland), and PBM were separated by density-gradient centrifugation over Ficoll-Hypaque PLUS (GE Healthcare, Uppsala, Sweden), recovered, washed twice and suspended in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum and 100 μg/ml penicillin–streptomycin (cRPMI). Cells were plated onto cell-culture flasks and incubated for 1 h, and then adherent PBM were recovered. Isolated monocytes gave rise to immature myeloid DC after culture for 7 days in cRPMI containing 50 ng/ml human recombinant GM-CSF and 30 ng/ml and human recombinant IL-4, or to MDM after culture for 5 days in cRPMI containing 50 ng/ml human recombinant M-CSF. After 7 and 5 days of incubation the percentage of immature DC ($CD1^+$, $CD40^+$, HLA DR^+, $Fc\gamma RII^-$ and $Fc\gamma RIII^-$) and MDM yields were 95% and 98% respectively.

2.3. C. albicans culture

C. albicans (CAF2-1 strain) was routinely maintained on Sabouraud 2% glucose agar. For evaluation of DC and MDM fungicidal activity, blastospores were harvested, washed twice, and diluted to the desired concentration. For all other experimental purposes, yeast cells were heat-inactivated at 56 °C for 30 min.

2.4. In vitro C. albicans binding and phagocytosis

For *C. albicans* binding, DC or MDM (1×10^5 cells/200 μl) were incubated for 30 min at 37 °C in 5% CO_2 with or without 2 μg/ml Dec1Ab or IrrAb as a negative control. DC or MDM were incubated with yeasts (5×10^5) in cRPMI for 20 min for binding or for two hours for phagocytosis, washed twice with PBS, transferred onto glass slides and stained with May Grünwald–Giemsa before examination under light microscopy. Alternatively, *C. albicans* binding and phagocytosis were performed by flow cytometry. Briefly, heat-killed yeasts were suspended in PBS at a density of 10^8 yeasts/ml. Cells were labeled with fluorescein isothiocyanate (FITC, Sigma) at 1 μg/ml in PBS at 22 °C for 10 min. Labeled yeasts (10^7) were incubated with DC (10^6) at 37 °C for 2 h. Phagocytosis was arrested by adding 1 ml ice-cold PBS to the suspension. Trypan blue (200 μg/ml, Sigma) was added to each sample and incubated for 10 min to quench fluorescence of non-internalized fungi. Unbound trypan blue was then removed by centrifugation and the percentage of phagocytic cells was determined by flow cytometry.

2.5. In vitro fungicidal activity

C. albicans blastospores (2×10^4) were plated in 96-well plates and incubated for 6 h (37 °C, 5% CO_2) with DC or