Immunobiology 220 (2015) 1093–1100

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Recognition of *Candida albicans* by Dectin-1 induces mast cell activation

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ARTICLE INFO

Article history:
Received 17 February 2015
Received in revised form 10 March 2015
Accepted 1 May 2015
Available online 11 May 2015

Keywords:
Mast cell
Candida albicans
Dectin-1
Cytokines
Chemokines

ABSTRACT

Mast cells are crucial elements of the innate immune response. They reside in tissues that are commonly exposed to the external environment, such as the skin and mucosae, where they can rapidly detect the presence of pathogens and mount a potent inflammatory response that recruits other cellular effectors of the immune response. The contribution of mast cells to the immune response to viruses, bacteria, protozoa and multicellular parasites is well established, but there is scarce information about the role of these cells in fungal infections. In this study, we analyzed if mast cells are activated by *Candida albicans* and if the C-type lectin receptor Dectin-1 is involved in its recognition. We found that both yeasts and hyphae of *C. albicans*-induced mast cell degranulation and production of TNF-α, IL-6, IL-10, CCL3 and CCL4, while only yeasts were able to induce IL-1β. Mast cells also produced ROS after stimulation with both dimorphic phases of *C. albicans*. When mast cells were activated with yeasts and hyphae, they showed decreased expression of IκBα and increased presence of phosphorylated Syk. Blockade of the receptor Dectin-1, but not Toll-like receptor 2, decreased TNF-α production by mast cell in response to *C. albicans*. These results indicate that mast cells are capable of sensing the two phases of *C. albicans*, and suggest that mast cells participate as an early inductor of inflammation during the early innate immune response to this fungus.

Introduction

Mast cells have been traditionally considered as fundamental initiators of inflammation during type I hypersensitivity, but more recent evidence indicates that mast cells are also crucial players in many biological processes (Galli and Tsai, 2008). One example is the participation of mast cells during the innate immune response to different pathogens (St John and Abraham, 2013). Mast cells are located strategically in the skin and mucosae, which makes them one of the first cell populations that are able to detect invading pathogens. Moreover, mast cells are equipped with a vast array of Pattern Recognition Receptors (PRR) including members of the Toll-like receptors (TLR), Nod-like receptors (NLR), C-type lectin receptors (CLR), RIG-I-like receptors (RLR) and scavenger receptors (Campillo-Navarro et al., 2014). Mast cell activation leads to a rapid release of preformed mediators through degranulation, followed by the production of different cytokines and chemokines that modulate the immune response. Several studies demonstrate that mast cells are essential for the early mounting of innate immune response to viruses (*e.g.* dengue virus and cytomegalovirus) (St John et al., 2013; Ebert et al., 2014), bacteria (*e.g. Klebsiella pneumoniae* and *Francisella tularensis*) (Malaviya et al., 1996; Ketavarapu et al., 2008), protozoa (*e.g. Leishmania major*, *Plasmodium berghei*) (Maurer et al., 2006; Furuta et al., 2006) and multicellular parasites (*e.g. Trichinella spiralis* and *Nippostrongylus brasiliensis*) (Woodbury et al., 1984; Knight et al., 2000); in response to these pathogens, mast cells produce cytokines and chemokines that recruit other innate immune cells, that leads to pathogen control (St John and

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http://dx.doi.org/10.1016/j.imbio.2015.05.005
0171-2985/

Abraham, 2013). The information about the role of mast cells during fungal infections is scarce (Saluja et al., 2012). Two studies showed that mast cells are activated *in vitro* by *Aspergillus fumigatus* and *Malassezia sympodalis* (Urb et al., 2009; Selander et al., 2009), and recent reports indicate that mast cells are activated by *Sporothrix schenckii*, a dimorphic fungus that causes sporotrichosis (Romo-Lozano et al., 2012; Romo-Lozano et al., 2014).

Candida albicans is an opportunistic dimorphic fungus that causes mucocutaneous and systemic candidiasis. *C. albicans* usually colonizes humans since birth and persist during the rest of their life as a commensal in the skin and the oral, gastrointestinal and vaginal mucosae (Netea et al., 2008). The immune response plays an essential role in the control of opportunistic infections by *C. albicans*, as alterations of the anatomic barriers or immunosuppression favors the development of the disease (Shoham and Levitz, 2005). Neutrophils and macrophages are considered as central components of the innate immune response to *C. albicans*, because of their phagocytic activity. These cells usually recognize *C. albicans* through different PRR, including TLR-2, TLR-4, mannose receptor, Dectin-1, Dectin-2, DC-SIGN and others (Miramon et al., 2013).

Dectin-1 is a type II transmembrane receptor that recognizes β-glucan (Brown and Gordon, 2001) and belongs to the CLR family. Several fungi are recognized by Dectin-1, including *C. albicans* (Drummond and Brown, 2011). Triggering of Dectin-1 initiates a signaling pathway characterized by Syk phosphorylation that leads to NFκB activation through CARD9 (Rogers et al., 2005; LeibundGut-Landmann et al., 2007). *In vitro* activation through Dectin-1 promotes diverse cellular responses such as the generation of reactive oxygen species (ROS) (Underhill et al., 2005), phagocytosis (Herre et al., 2004) and the production of several cytokines and chemokines (Drummond and Brown, 2011). Furthermore, mice deficient in Dectin-1 are unable to control the dissemination of *C. albicans* and have an increased susceptibility to infection by this fungus (Taylor et al., 2007). Dendritic cells also recognize *C. albicans* through Dectin-1; these cells prime T cell responses toward a Th17 profile, which is considered essential for infection containment (LeibundGut-Landmann et al., 2007; Cheng et al., 2011).

Considering that mast cells can degranulate in response to glycoproteins from *C. albicans* and kill yeasts in the extracellular environment (Nosal et al., 1974; Trevisan et al., 2014), and that mast cells express Dectin-1 (Olynych et al., 2006; Yang and Marshall, 2009), we decided to test the hypothesis if *C. albicans* induces cytokine, chemokine and ROS production by mast cells, and if Dectin-1 was involved in this activation.

Material and methods

Candida albicans *culture*

C. albicans strain 3147 (ATCC 10231, USA) was grown at 28 °C in Sabouraud dextrose medium (Difco, USA) on a rotating drum for 24 h. Yeast cells were aliquoted and stored at −70 °C until use. *C. albicans* viability was determined after serial dilutions and seeding in plates with Sabouraud agar (Difco, USA). CFU were determined after overnight incubation at 28 °C. Hyphae induction was carried out as previously described (Bassilana et al., 2005), briefly *C. albicans* cells were incubated in RPMI 1640 medium (Gibco, USA) supplemented with 50% fetal bovine serum (FBS, Gibco, USA) for 2 h at 37 °C. Where indicated, fungi were heat-killed by incubation at 65 °C for 2 h in a water bath.

Mast cells

Peritoneal mast cells (PMC) were obtained by lavage of the peritoneal cavity of male Sprague Dawley rats with HEPES-buffered Tyrode's solution. PMC were further purified on a discontinuous Percoll gradient, which yielded a purity >98%, as assessed by toluidine blue staining.

Bone marrow derived mast cells (BMMC) cells were obtained as previously described (Chacon-Salinas et al., 2014); briefly, the bone marrow from the femurs and tibias of 6–10-week-old BALB/c mice was disaggregated and cultured at a concentration of 1×10^6 cells/ml in RPMI 1640 supplemented with 10% of FBS and 10 ng/ml of murine recombinant IL-3 and 10 ng/ml of SCF (BioLegend USA). Non-adherent cells were transferred to fresh culture medium twice a week for 4–8 weeks, at which point mast cell purity was >90% according to CD117 and FcεRIα measured by flow cytometry.

The protocol for PMC obtainment from rats was reviewed and approved by the Local Comitte for Research and Ethics in Research of the Mexican Institute of Social Security (IMSS), while the protocols for BMMC obtainment from mice was reviewed and approved by the Comitee for Ethics in Research of ENCB, IPN.

Mast cell degranulation assays

BMMC were washed three times with Hanks Balanced Salt Solution (HBSS) (Gibco, USA) pre-warmed at 37 °C. 1×10^6 cells/mL were seeded in a 5 ml round bottom polystyrene tube (Corning, Mexico) and stimulated with either live yeasts or hyphae of *C. albicans* (MOI 1). After 30 min, cells were washed two times with PBS-BSA 0.1% and labeled with anti-CD107a/PE (BioLegend, USA, clone 1D4B) and with anti-FcεRIα Alexa 647 (eBioscience, USA, clone MAR-1). After washing the cells, surface staining was measured with a FACSCalibur flow cytometer (BD Biosciences, USA), and the results were analyzed with FlowJo (Tree Star, USA).

β-Hexosaminidase secretion assay was performed as previously described (Yepez-Mulia et al., 2009); briefly, 5×10^5 PMC were stimulated with live *C. albicans* yeast or hyphae (MOI 10), or with 1 μg/mL Compound 48/80 (Sigma, USA). After 30 min, supernatants were collected and β-hexosaminidase activity was measured in medium and cell lysates by the hydrolysis of its substrate 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide (Sigma, USA). Results are presented as the percentage of intracellular β-hexosaminidase that was released into the medium after correcting for spontaneous release.

Quantification of cytokines, chemokines and ROS

1×10^6 BMMC were seeded on 24 well-plates and left unstimulated or stimulated with live *C. albicans* yeasts or hyphae (MOI 1) or PMA (Sigma USA) 125 ng/ml plus 5 μM calcium ionophore A23187 (Sigma USA), during 1, 6 and 24 h. Supernatants were collected, cleared and evaluated for murine TNF-α (BD Bioscience, USA), IL-1β (BD Bioscience, USA), IL-6 (BD Bioscience, USA), IL-10 (Biolegend, USA), MIP-1α (CCL3) (Peprotech, USA), MIP-1β (CCL4) (Peprotech, USA) by ELISA, according to manufacturers' instructions.

To evaluate ROS production, 5×10^5 BMMC were resuspended in 1 ml of HBSS and labeled with 5 μM DCFH-DA (Molecular Probes, USA) for 15 min at 37 °C. After washing, cells were left unstimulated or were stimulated for 2 h with live *C. albicans* yeasts or hyphae (MOI 10), or with 125 ng/ml of PMA (Sigma, USA), at 37 °C. ROS generation was detected in FL-1 channel in a FACSCalibur flow cytometer (BD Biosciences, USA), and the results were analyzed with FlowJo (Tree Star, USA).