Immunology Letters 168 (2015) 13–21

Contents lists available at ScienceDirect

Immunology Letters

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

Secreted aspartic protease 2 of *Candida albicans* inactivates factor H and the macrophage factor H-receptors CR3 (CD11b/CD18) and CR4 (CD11c/CD18)

Eliška Svoboda[a], Andrea E. Schneider[b], Noémi Sándor[c], Ulrich Lermann[d], Peter Staib[d,e], Mariann Kremlitzka[c], Zsuzsa Bajtay[f], Dagmar Barz[g], Anna Erdei[c,f], Mihály Józsi[a,b,*]

[a] *Junior Research Group Cellular Immunobiology, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany*
[b] *MTA-ELTE "Lendület" Complement Research Group, Department of Immunology, Eötvös Loránd University, Budapest, Hungary*
[c] *MTA-ELTE Immunology Research Group, Department of Immunology, Eötvös Loránd University, Budapest, Hungary*
[d] *Junior Research Group Fundamental Molecular Biology of Pathogenic Fungi, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute, Jena, Germany*
[e] *Research and Development, Kneipp GmbH, Würzburg, Germany*
[f] *Department of Immunology, Eötvös Loránd University, Budapest, Hungary*
[g] *Institute for Transfusion Medicine, University Hospital of Jena, Germany*

ARTICLE INFO

Article history:
Received 4 July 2015
Accepted 19 August 2015
Available online 22 August 2015

Keywords:
Candida albicans
Complement
CR3
Factor H
Fungal pathogen
Immune evasion
Protease
Sap2

ABSTRACT

The opportunistic pathogenic yeast *Candida albicans* employs several mechanisms to interfere with the human complement system. This includes the acquisition of host complement regulators, the release of molecules that scavenge complement proteins or block cellular receptors, and the secretion of proteases that inactivate complement components. Secreted aspartic protease 2 (Sap2) was previously shown to cleave C3b, C4b and C5. *C. albicans* also recruits the complement inhibitor factor H (FH), but yeast-bound FH can enhance the antifungal activity of human neutrophils via binding to complement receptor type 3 (CR3). In this study, we characterized FH binding to human monocyte-derived macrophages. Inhibition studies with antibodies and siRNA targeting CR3 (CD11b/CD18) and CR4 (CD11c/CD18), as well as analysis of colocalization of FH with these integrins indicated that both function as FH receptors on macrophages. Preincubation of *C. albicans* yeast cells with FH induced increased production of IL-1β and IL-6 in macrophages. Furthermore, FH enhanced zymosan-induced production of these cytokines. *C. albicans* Sap2 cleaved FH, diminishing its complement regulatory activity, and Sap2-treatment resulted in less detectable CR3 and CR4 on macrophages. These data show that FH enhances the activation of human macrophages when bound on *C. albicans.* However, the fungus can inactivate both FH and its receptors on macrophages by secreting Sap2, which may represent an additional means for *C. albicans* to evade the host innate immune system.

1. Introduction

Candida albicans is an opportunistic fungal pathogen in humans that is part of the mucosal microflora in the majority of the human population. It can cause a range of superficial and invasive life-threatening infections in individuals with a compromised immune system [5]. *C. albicans* possesses several virulence factors that assist its success to persist and cause infection in the human host. One of the virulence traits of *C. albicans* is the release of proteolytic enzymes, such as secreted aspartyl proteases (Sap), which cleave fluid-phase and extracellular matrix proteins and thus cause tissue damage and facilitate infection [41]. Sap2 is a secreted enzyme essential for *C. albicans* growth in a protein-rich environment that cleaves peptide bonds between hydrophobic amino acids [16]. In the human host, Sap2 cleaves various proteins of the extracellular matrix, antimicrobial peptides, and the complement components C3b, C4b and C5, and contributes to fungal virulence [40,27,12].

Abbreviations: CCP, complement control protein domain; CR3, complement receptor type 3 (CD11b/CD18); CR4, complement receptor type 4 (CD11c/CD18); FH, factor H; MDM, monocyte-derived macrophages; Sap2, secreted aspartic protease 2.

* Corresponding author at: MTA-ELTE "Lendület" Complement Research Group, Department of Immunology, Eötvös Loránd University, Pázmány Péter sétány 1/c, 1117 Budapest, Hungary. Fax: +36 1 381 2176.
E-mail address: mihaly.jozsi@gmx.net (M. Józsi).

http://dx.doi.org/10.1016/j.imlet.2015.08.009
0165-2478/

The complement system is a crucial humoral component of innate immunity, which serves the immediate protection against intruding microorganisms [34]. Beside its role in pathogen elimination mediated directly by formation of the lytic terminal complement complex C5b-9 or indirectly by opsonization (mainly with C3b and C4b complement fragments), the complement system participates in the disposal of immune complexes and apoptotic cells, and the modulation of activation of the cellular components of the immune system [34].

Because of its destructive potential for the host, the complement system encompasses several regulator molecules. Factor H (FH) is the major soluble regulator of the alternative pathway, which acts in body fluids as well as at cellular surfaces by assisting the degradation of C3b by factor I (termed cofactor activity) and by preventing the formation and accelerating the decay of the alternative pathway C3-convertase. FH consists of 20 complement control protein (CCP) domains, of which the N-terminal CCPs 1-4 are responsible for both the cofactor- and decay accelerating activity of FH, and the C-terminal CCPs 19-20 mediate host surface binding [20].

Several pathogenic and non-pathogenic microbes, including various fungi, were shown to sequester host complement inhibitor molecules, such as FH, to escape from the complement system [22]. Due to the thick fungal cell wall, complement cannot harm pathogenic fungi directly by lysis. However, the activation of complement can lead to opsonization of the fungal surface with C3b and C4b, and result in the generation of chemotactic and pro-inflammatory activation fragments, such as C3a and C5a [45,7], which together facilitate the elimination of the pathogen by attracting neutrophils and macrophages and enhancing opsonophagocytosis. In order to evade these complement-mediated defence processes, some fungi bind complement regulators, such as FH and C4b-binding protein, through a variety of surface-bound and secreted proteins [33,54,25] and thus restrict complement activation in their environment. In addition, FH was reported to influence cellular adhesion and/or antifungal responses. Due to its binding via various domains, FH can bridge different pathogens, such as *Streptococcus pneumoniae*, *Neisseria gonorrhoeae* and *C. albicans*, with the CR3 complement receptor and enhance cellular invasion or uptake of these pathogens and the immune response of the host cells [2,3,23]. FH, when bound to the surface of *C. albicans,* was shown to mediate fungal recognition by neutrophils via primarily CR3, and increase the production of lactoferrin and reactive oxygen species by these host cells [23].

FH binding to various immune cells has been described; in some cases, however, neither the nature of the FH receptor, nor the relevance of this interaction is known, such as in the case of B lymphocytes [21,10]. FH has been identified as a ligand of L-selectin on leukocytes [26]. FH binds to neutrophils via complement receptor 3 (CR3; CD11b/CD18) [6,9] and this interaction is mediated through the CCP7 and CCPs 19-20 of FH [23]. It is also to be noted that neutrophils express a unique, fucosylated form of CR3, which may influence ligand interactions of CR3 [53]. Purified FH has been shown to be chemotactic for human monocytes at a nanomolar concentration [31] and the coincubation of human monocytes with FH stimulated the secretion of reactive oxygen species and IL-1β in a dose-dependent manner [42,17]. In addition, macrophages stimulated with FH exhibited an elevated production of tromboxane and prostaglandine E [13]. FH has recently been shown to bind in part to CR3 on monocytes and down-modulate the C1q-mediated uptake of apoptotic cells [19], and it was also shown to enhance the phagocytosis of apoptotic particles and decrease the release of IL-8 and TNF-α by macrophages [29]. Nevertheless, the nature of the FH receptor on mononuclear phagocytes and the role of pathogen-bound FH in the response of macrophages are poorly characterized.

The tissue-resident mononuclear phagocytes and the recruited neutrophils represent the first and major defence against *C. albicans* [4,50]. The fungus is recognized by the phagocytes either directly through pattern recognition receptors or indirectly, after opsonization, via opsonic receptors. The CR3 and the related CR4 (CD11c/CD18) receptors are major opsonic receptors, which bind the C3b cleavage product iC3b. CR3 can also bind directly to fungal surface moieties, such as beta-glucan and pH-regulated antigen 1, which interactions are important in the antifungal innate cellular response [52,43,44,24].

Macrophages express more CR4 in comparison with neutrophils, however, the role of FH-CR3/CR4 interaction in the context of antifungal response of macrophages has not been studied yet. Therefore, the aim of this study was to reveal the role of CR3 and CR4 in FH binding on human macrophages and to study whether Sap2 can facilitate the evasion of the FH-CR3/CR4 mediated activation of macrophages.

2. Materials and methods

2.1. Ethical approval

The studies were performed with approval of the Research Ethics Committee of the Medical Faculty of Friedrich Schiller University, Jena (permission number 2268-04/08) and by the respective Hungarian authorities (permission number ETT TUKEB 838/PI/12.). Informed consent for the use of blood samples was obtained according to the Declaration of Helsinki.

2.2. Materials

Purified human FH and the goat FH-specific polyclonal antibody were purchased from Merck (Schwalbach, Gemany). The anti-CD11b (clone ICRF44), anti-CD11c (clone B-ly6), anti-CD18 (clone L130) monoclonal antibodies and isotype controls were purchased from BD Biosciences (Heidelberg, Germany). HRP-conjugated rabbit anti-goat IgG, FITC-conjugated rabbit anti-goat IgG and $F(ab')_2$ fragments of goat anti-mouse IgG were obtained from Dako (Hamburg, Germany).

2.3. Human cell culture

THP-1 macrophages were differentiated from THP-1 monocytic cells (DSMZ, Braunschweig, Germany) by incubation with 10 nM PMA in RPMI 1640 medium (LONZA, Wuppertal, Germany) containing 10% FCS (PAA, Cölbe, Germany) for 24 h.

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque (GE Healthcare, Freiburg, Germany) density gradient separation and erythrocytes were lysed using a hypotonic salt solution. Monocytes were obtained from PBMC by positive selection with CD14 magnetic beads (Miltenyi Biotech, Bergisch Gladbach, Germany), following the manufacturer's instructions. Briefly, PBMC were resuspended in MACS buffer (DPBS with 0.5% BSA and 2 mM EDTA) containing CD14 microbeads. After incubation for 30 min at 4 °C, cells were washed and resuspended in MACS buffer and loaded onto MiniMACS LS Separation Columns (Miltenyi Biotech). After washing out of non-labeled cells with MACS buffer, monocytes were eluted and the purity of CD14-positive monocytes was determined by flow cytometry.

Monocyte derived macrophages (MDM) were obtained by culturing isolated monocytes for 7 days in X-VIVO 15 medium supplemented with 10% heat-inactivated FCS (PAA), 2 mM ultraglutamine (Lonza) and 50 μg/ml gentamicin sulfate (Lonza) and by addition of 500 IU/ml GM-CSF (Immunotools, Friesoythe, Germany) every 48 h. MDM were resuspended at 2×10^6 cells/ml in serum-free X-VIVO 15 medium and cultivated at 37 °C in a humidified atmosphere containing 5% CO_2.