Chapter

Responses of White Blood Cells to Killed *Candida albicans* as a Preventive Strategy

Ahmad Ibrahim

Abstract

C. albicans is by far the most common Candida species causing infection in humans which include superficial and a life- threatening systemic infections. Despite the public health significance of candida infections, phenotypic switching of *C. albicans*, slow mycological diagnosis, limitation of use of antifungal agents due to toxicity, high cost and emergence of resistance have impeded effective treatment. Therefore, a need for safe and potent strategy to prevent this disease is necessary. This chapter discusses the roles of white blood cells as the first line defense mechanism against inactivated *C. albicans*.

Keywords: white blood cells, *Candida albicans*, immune response

1. Introduction

Fungal infections are a serious public health concerns, particularly with the growing number of immunocompromised individuals. *C. albicans* amongst other fungal species has been identified as one of the leading cause of infections in recent times [1]. Candidemia and Candidiasis account for 50% and 70% prevalence infections in human and has caused a great deal of morbidity and mortality largely because of the polymorphic nature of *C. albicans*. Also, factors such as toxicity of antifungal drugs, drug resistance, limited arsenal of antifungal drugs, slow mycological diagnosis, variable drug bioavailability in immune-compromised patients and drug interactions have truncated every efforts being made at mitigating the prevalence and its consequential effects. These challenges have led to the several attempts at developing a viable preventive option for candida infections. The cellular surface of *C. albicans* is a predominant source of immuno-stimulatory antigens [2] comprising of 90% carbohydrates and 10% proteins [3] and hence making carbohydrates dominate immune recognition while the proteins exhibit the key role of adhesive interactions with the host cellular surfaces. Therefore, complete inactivation of the pathogen amongst many strategies such as using the genetic material, a specific protein on the cell surface or attenuation to prevent candidiasis has been attempted. The killed *C. albicans* has lost its ability to infect their hosts but can stimulate enough immunological responses for the host protection. White blood cells response against *C. albicans* is initiated within the first few hours of inoculation or infection.

Therefore during host – killed *C. albicans* interaction, the cell surface molecules trigger and modulate cell mediated (T cells) and innate immune cells (macrophages, neutrophils and natural killer cells) to respond appropriately. These cells

are considered to be the first line and most important defense mechanism against Candidiasis [4] and consequently induced a strong response against the pathogen [5]. These responses function synergistically, co-operate and modulate each other with the final goal of fighting infection (4).

2. Recognition of *C. albicans* in mucosal surfaces

The epithelial cells represent the first line of defense against Candida infection on mucosal surfaces. As the predominant cells in the innate immunity of the host, epithelial cells express pattern recognition receptors, which recognize *C. albicans* by interacting with pathogen-associated molecular patterns on the fungal cells. However, there are three major groups of these receptors (Toll-like receptors, C-type lectin receptors and nod-like receptors) but only certain Toll-like receptors and C-type lectin receptors on epithelial surfaces recognize *C. albicans*. In addition to pattern recognition receptors, other cell-surface proteins, such as E-cadherin and Epidermal Growth Factor Receptor, can also recognize Candida and these are unsurprisingly implicated in Candida adherence and endocytosis [6, 7].

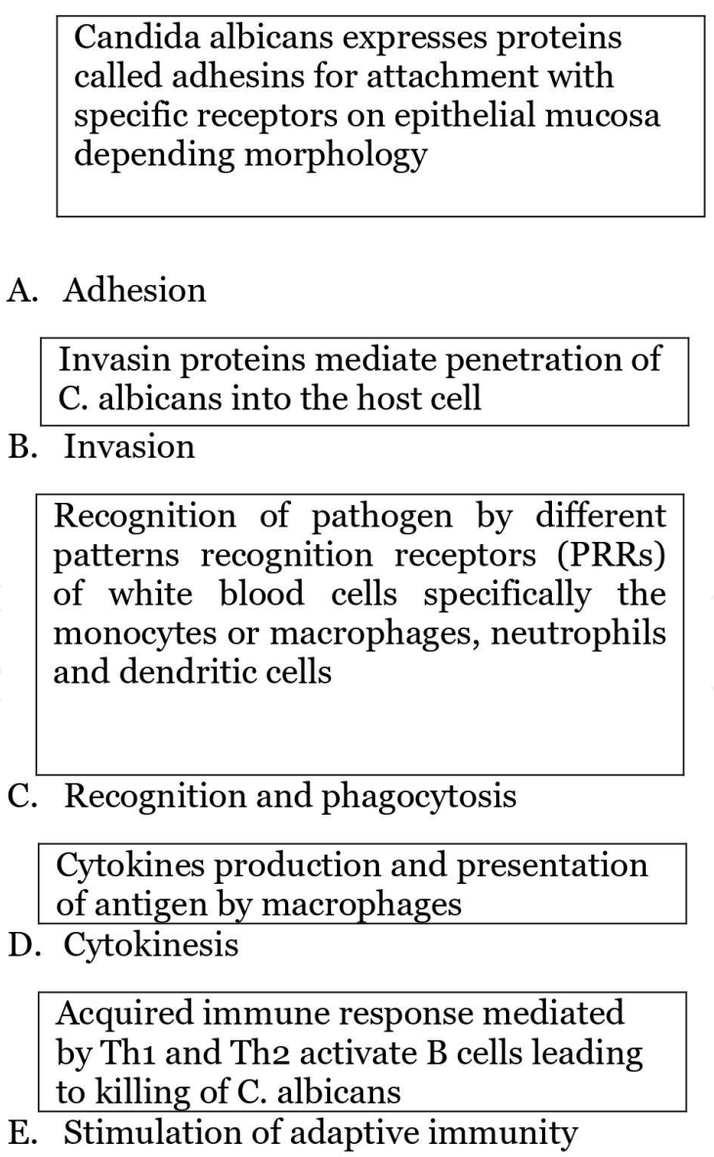

Figure 1.
Summary of host immune response against C. albicans.

2.1 Host immune response

The cell surface receptors on *C. albicans* initiate adhesive interactions and invade the host cell using a series of proteins including adhesins and invasins. These immunodominant factors would trigger and stimulate a complex interplay of natural and adaptive immunity, posing interesting immunological response to the host. Cell-mediated (T cells) and innate immunity (macrophages, neutrophils and natural killer cells) are considered to be the most important line of defense against candidiasis [4] as they are recruited into the site of infection to exert protective effects. These include phagocytosis and antigen presentation, opsonization and production of chemicals for effective killing of the microbial cells. It must be emphasized therefore, that these responses comprise of different arms of the immune system (innate, cell-mediated and antibody-mediated) as shown in **Figure 1**.

2.1.1 Innate immunity

White blood cells are produced and derived from multi-potent cells in the bone marrow known as hematopoietic stem cells and are found throughout the body, including the blood and lymphatic system [8]. Five individual types of white blood cells namely neutrophil, monocytes, lymphocytes, basophils, eosinophils [9] are involved in sustaining immunity [10].

Innate immune response is the dominant protective mechanism against disseminated candidiasis [11] and host defense against fungal infection depends on elimination of the fungi by phagocytic cells of the innate immune system, especially neutrophils and macrophages [12] at the initiation of infection before other immune cells are mobilized. Therefore, white blood cells are used to assess the working condition of body's immune system, to determine an active or chronic infection, identify the type of infection and also point to an allergic response or inflammation in the body [9].

Hence, quantitative and qualitative abnormalities of these immune cells are indications to different physiological conditions and particularly neutrophils and monocytes are associated with systemic candidiasis.

2.1.1.1 Neutrophils

Neutrophils or Polymorphonuclear leukocytes are the predominant phagocytic immune cells that play a major role against *C. albicans* infection. These cells activate various antimicrobial mechanisms in addition to phagocytosis, such as producing reactive oxygen species, the release of granular enzymes and antimicrobial proteins [13]. In addition, a neutrophil extracellular trap composed of a neutrophil chromatin is another significant protective strategy deployed by the host against fungal infections [14]. Neutrophils and monocytes damage and kill yeast cells of *C. albicans*, hyphae and pseudohyphae [11] by recognizing and engulfing opsonized and non-opsonized yeast cells via cell-surface pattern recognition receptors. However, the large size of Candida hyphae and pseudohyphae may preclude phagocytosis and thus the need for several phagocytes to collaborate and affect extracellular killing [15].

2.1.1.2 Monocytes

Neither dead cell debris nor attacking microorganisms can be dealt with effectively by the neutrophils [16]. Monocytes and their derivatives, including macrophages and dendritic cells, play diverse roles in the response to fungal pathogens

by sensing fungi and triggering signaling pathways that mediate direct effects like phagocytosis, cytokine production and presentation of fungal antigens to elicit adaptive immune response [17].

- **Phagocytosis**

  In phagocytosis, fungi can be eliminated in monocytes and their derivatives in the phagolysosome. This is an acidified compartment that contains enzymes such as Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase (generates reactive oxygen species), and inducible nitric oxide synthase (produce Nitrogen IV Oxide and reactive nitrogen species) that can sequester nutrients and in response to pro-inflammatory stimuli [18]. This fungal killing may be sufficient to halt the progression of infection, but it can also provide fungal antigens that can be used to initiate the adaptive immune response to ensure sterilizing immunity. Fungal uptake is not always beneficial to the host, however, as some fungi have adapted to the harsh environment in the phagolysosome or can subvert monocytes to enable fungal persistence and proliferation [17].

- **Production of Cytokines**

  Cytokines are a group of low molecular weight proteins that act as a mediator between cells and are produced by white blood cells and other non-immune cells in response to stimuli [19].

  Monocytes and their derivative cells can produce chemokines, pro-inflammatory, anti-inflammatory and pleiotropic cytokines [20, 21]. These cytokines and chemokine secretion is important for the development of both the innate and adaptive immune response to fungal pathogens and can influence the activation and recruitment of other immune cells and the polarization of the adaptive immune response [17]. Under normal physiological condition, cytokines are not detectable or are present at low levels in body fluids or tissues because they are only produced when required in immune responses [19]. Therefore, an elevated levels or unregulated production of cytokines may be associated with inflammation or disease pathogenesis [22].

- **Presentation of Antigens**

  Antigen-Presenting Cells (APCs) are cells that can process a protein antigen, break it into peptides, and present it in conjunction with class II Major Histocompatibility Complex (MHC) molecules on the cell surface where it may interact with appropriate T cell receptors. Monocytes and their derivative are professional APCs and are amongst the principal antigen-presenting cells for T cells [23]. Antigen-Presenting Cells are critical for the initiation of adaptive immune responses and for maintenance of peripheral tolerance [24]. Dendritic cells serve as the connection between innate and acquired immunity and morphological characteristics of *C. albicans* dictates the specific immune response [25]. For example, the interaction of dendritic cells with yeast cells or pseudohyphal sensitize different receptors. Therefore, when yeast form of *C. albicans* is engulf by dendritic cells, differentiation of CD4+ cells into T-helper 1 cells is induced, while dendritic cells stimulated by the pseudohyphal form induce a T- helper 2 response. The response produced by

T- helper 1 cells is linked with protection of the host against fungal infection while for T- helper 2, responses are related to the ability of microorganisms to escape or suppress the host's immune response. Nonetheless, T-helper 1 and T-helper 2 responses activate B cells and leads to maturation of other phagocytic cells [23].

2.1.1.3 Eosinophils and basophils

Eosinophils and Basophils are both granulocytes characterized by their content of intracellular granules. These cells become especially active during an allergic response and are responsible for releasing histamine [9]. Fungi also represent a source of major allergens.

While the roles of eosinophils in an allergic disease associated with fungal sensitization is still debatable or even poorly understood, their contributions to remodeling are more accepted.

2.2 Inactivation of *Candida albicans*

The commonest methods for inactivation of *C. albicans*, in the preparation of an immune-based prevention of *C. albicans* infection include using of heat and a source of UV. According to Evron, [26], whole *C. albican* cells suspended in 0.85% sterile normal saline and heated at 65°C are inactive. While exposing *C. albicans* suspended in 0.85% sterile normal saline directly to a source of Ultraviolet radiation (UV) at a wavelength of 254 nm for 30 minutes inactivates the cells [27]. This inactivation renders the *C. albicans* non-viable to infect an intended host but retains the structural conformation of immunogenic components on the cell surface.

2.2.1 Response of antibodies to Killed C. albicans

Adherence of lymphocytes to a fungus is the first step in the direct lymphocyte-mediated anti- fungal effect against *C. albicans* [1]. Experimental study indicates that antibodies play an important role in host defense against disseminated candidiasis because individuals with defects in cell mediated immunity mechanisms are particularly prone to superficial but not disseminated candidiasis [28].

Therefore, humoral mediated immune response results in a significant elevated level of antibodies in Wister albino rats exposed to killed *C. albicans*. This could be as a result of recognition of the immunogenic proteins and glycoproteins on the cell surface and subsequent stimulation of memory cells to produce significant quantity of antibodies on a second encounter of similar antigens on killed *C. albicans* that are immunoprotective. According to Evron, [29], circulating antibodies in mice exposed to killed - *C. albicans* that are immunoprotective should be greater than 256 μg/m. Hence a concerted effort for more research to produce vaccines that can stimulate the release of even more antibodies in rats and subsequently in human are necessary.

2.2.2 Response of phagocytic cells (monocytes, macrophages and granulocytes)

Phagocytic cells such as the granulocytes and monocytes play an important role in cell-mediated immunity (T-cells and phagocytic cells such as monocytes, granulocytes) and so attacks the killed *C. albicans* in similar mechanism as though it is viable and infectious cells. These cells are the first line defense mechanism and are recruited in large quantity in the first few days of injection of the killed *C. albicans*

to the Wister rats. Granulocytes being components of white blood cells are recruited as innate immune response to engulf the killed *C. albicans* [29]. However, viable *C. albicans* have the ability of switching or morphogenesis which allows them to escape phagocytosis by piercing of phagocytes and subsequent killing of phagocytic cells, leading to a decrease in circulating granulocytes in blood [30]. Consequently, contributing to a large extent the impeding factor for availability of an effective vaccine.

2.2.3 Delayed-type hypersensitivity

Delayed-Type Hypersensitivity reaction is initiated when antigens are presented by antigen presenting cells (i.e. langerhans cells) to sensitized memory T cells. The antigen presentation and subsequent T cell activation elicit an influx of macrophages, monocytes and lymphocytes at the site of antigen exposure. At the onset of delayed-type hypersensitivity reaction, verso-permeability is increased so that additional cellular components migrate into the local site of antigen presentation [31] and this explains the swelling at the site of injection of killed *C. albicans*. Therefore, inactivated *C. albicans* have an immune-stimulatory property.

3. Conclusion

The need for appropriate immuno-prophylaxis or immunotherapy against candidiasis is readily apparent. Therefore, the relationship between killed *Candida albicans* and the hosts' white blood cells in terms of recognition and response clearly suggest an interesting immunoprotection against viable *C. albicans.*

A safe and effective therapeutic alternatives to combat these infections and to eliminate potential problems of toxicity and emergence of resistance to the limited options of antifungal drugs is needed [32]. Therefore, killed *C. albicans* is an immune-based prophylactic and therapeutic approach [33] which represents novel option against *C. albicans* infections [32].

Thanks

I want to specially thank my brother, Muhammad I. Odaki for his tremendous support.

Author details

Ahmad Ibrahim
Department of Biochemistry, Federal University Lokoja - Kogi State, Nigeria

*Address all correspondence to: ahmadibrahim337@yahoo.com

References

[1] Forsyth C. B. and Mathews, H. C. (2002). Lymphocytes adhesion to C. albicans. 70(2): 517-527.

[2] Martınez, J.P., Gil, M.L., Lopez-Ribot, J.L. and Chaffin, W.L. (1998) Serologic response to cell wall mannoproteins and proteins of Candida albicans. Clin. Microbiol. Rev. 11, 121-141.

[3] Staib P, Morschhäuser J. (2007). "Chlamydospore formation in Candida albicans and *Candida dubliniensis* an enigmatic developmental programme". Mycoses 50 (1): 1-12. PMID 17302741.

[4] Levitz, S.M. (1992). Overview of host defenses in fungal infections. Clin. Infect. Dis. 14 (Suppl 1), S37–S42.

[5] Casadevall, A., Cassone, A., Bistoni, F., Cutler, J.E., Magliani, W., Murphy, J.W., et al., (1998). Antibody and/or cell-mediated immunity, protective mechanisms in fungal disease: an ongoing dilemma or an unnecessary dispute? Med. Mycol. 36, 95-105.

[6] Phan QT, Myers CL, Fu Y, Sheppard DC, Yeaman MR, Welch WH, et al., (2007). Als3 is a Candida albicans invasin that binds to cadherins and induces endocytosis by host cells. PLoS Biol. 5:e64.

[7] Sun JN, Solis NV, Phan QT, Bajwa JS, Kashleva H, Thompson A, Liu Y, Dongari-Bagtzoglou A, Edgerton M, Filler SG (2010). Host cell invasion and virulence mediated by Candida albicans Ssa1.PLoS Pathog. 6:e1001181.

[8] Maton, D., Hopkins, J., McLaughlin, Ch. W., Johnson, S., Warner,et al.,(1997). Human Biology and Health. Englewood Cliffs, New Jersey, US: Prentice Hall. ISBN 0-13-981176-1

[9] D'Aquila R., (2011) – How to Interpret Your Blood Tests: Part II. NYC Chiropractor – Applied Kinesiology. https://robdaquila.com/2011/03/15/how-to-interpret-your-blood-tests-part ii/received 15/03/2011.

[10] Diong, K., and Thompson, L. A., (2017). A methodical approach to interpreting the white blood cell parameters of the complete blood count. American Society of Clinical Laboratory Science. 30(3): 186-193

[11] Diamond, R.D., Clark, R.A. & Haudenschild, C.C. (1980). Damage to Candida albicans hyphae and pseudohyphae by the myeloperoxidase system and oxidative products of neutrophil metabolism in vitro. Journal of Clinical Investigation, 66, 908-917.

[12] Blanco JL, Garcia ME (2008). Immune response to fungal infections. Vet Immunol Immunopathol. ;125:47-70.

[13] Robinson JM (2008). Reactive oxygen species in phagocytic leukocytes. Histochem Cell Biol. 130:281-297.

[14] Urban CF, Reichard U, Brinkmann V, Zychlinsky A., (2006). Neutrophil extracellular traps capture and kill Candida albicans yeast and hyphal forms. Cell Microbiol. 8:668-676.

[15] Bellocchio, S., Montagnoli, C., Bozza, S., Gaziano, R., Rossi, G., Mambula, et al., (2004). The contribution of the Toll-like/IL-1 receptor superfamily to innate and adaptive immunity to fungal pathogens in vivo. Journal of Immunology, 172, 3059-3069.

[16] Sozzani, S.; Zhou, D.; Locati, M.; Bernasconi, S.; Luini, W.; Mantovani, A.; O'Flaherty, J. T. (1996). "Stimulating properties of 5-oxo-eicosanoids for human monocytes: synergism with monocyte chemotactic protein-1 and -3".

The Journal of Immunology. **157** (10): 4664-4671

[17] Heung, L.J. (2020). Monocytes and the host response to fungal pathogens. Front. Cell infect. Microbial. 10:34

[18] Uribe-Querol E., Rosales C. (2017). Control of phagocytosis by microbial pathogens. Front. Immunol. 8:1368. 10.3389/fimmu.2017.01368

[19] Chin, VK., Foong, KJ. Maha, A. Rusliza, B. Norhafizah, M. Chong, P.P (2014). Early expression of local cytokines during systemic *Candida albicans* infection in a murine intravenous challenge model, Pages: 869-874

[20] Carson W. E., Ross M. E., Baiocchi R. A., Marien M. J., Boiani N., Grabstein K., et al. (1995). Endogenous production of interleukin 15 by activated human monocytes is critical for optimal production of interferon-gamma by natural killer cells *in vitro*. J. Clin. Invest. 96, 2578-2582.

[21] Arango Duque G., Descoteaux A. (2014). Macrophage cytokines: involvement in immunity and infectious diseases. Front. Immunol. 5:491.

[22] Abbas AK, Lichtman AH and Pober JS: Cellular and Molecular Immunology. 3rd edition. W.B. Saunders; Philadelphia, PA: pp. 15-37. 1997

[23] Cruse, J. M., Lewis, R. E. & Wang, H. (2004). Antigen Presentation: Immunology Guide Book. Academic Press. 267-276

[24] Dana, R. & Hamrah, P., (2010). APCs in the Eye and Ocular Surface. Encyclopedia of the Eye. 120-127.

[25] Ranta, K. Nieminen, T. Saariaho et al., (2013). "Evaluation of fungal extracts to determine immunomodulatory properties," Journal of Investigational Allergology and Clinical Immunology, vol. 23, no. 4, pp. 226-233.

[26] Evron Ruth (1980). In Vitro Phagocytosis of *Candida albicans* by Peritoneal Mouse Macrophages. *infection and immunity*, p. 963-971 Vol. 28, No. 3. 0019-9567/80/06-0963/09$02.00/0

[27] Wheeler, RT., and Fink, G.R. (2006). A drug –sensitive genetic network masks fungi from the immune system. PLOS Pathogens, 0328-0338

[28] Maathew R and Burnie, (2001). Antifungal antibodies: a new approach to the treatment of systemic candidiasis. Current opinion in investigational drugs 2:472-476

[29] Aderem A. (2003). Phagocytosis and the Inflammatory Response. The Journal of Infectious Diseases, Volume 187, Issue Supplement_2, 15 June 2003, Pages S340-5, https://doi.org/10.1086/374747

[30] Uwamahoro, N., Verma-Gaur, J., Shen, H. H., Qu, Y., Lewis, R., Lu, J., Bambery K., *et al*., (2014). The pathogen C. albicans hijacks pyroptosis for escape from macrophages. American Society of Microbiology. 5(2): 1-11

[31] Jyonouchi Harumi, (2015). Delayed Type Hypersensitivity. Emedicine. medscape.com/article/886393-overview.

[32] Wang *X., Sui, X. L. E., & Jiang Y. (2015). Vaccinces in the treatment of invassive land disease*. Virulence *6(4):309-315*

[33] Deepe Jr., G.S. (1997). "Prospects for the development of fungal vaccines". Clin. Microbiol. Rev. 10:585-596.