

**Neutrophils: The Gatekeepers to Candidal Infection**Aliyu, M.<sup>1</sup>, Muhammed, Y.<sup>1</sup>, Azeez-Akande, O.<sup>1</sup> and Jibril, M.<sup>2\*</sup>

*Department of Medical Microbiology and Parasitology, Faculty of Clinical Science, College of Health Sciences, Bayero University, Kano, Nigeria*<sup>1</sup>, *Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, College of Natural and Pharmaceutical Sciences, Bayero University, Kano, Nigeria*<sup>2</sup>.

\*Author for Correspondence: +234-803-445-3990/murtalamj@gmail.com

**Summary**

Candida causes a spectrum of infection from mild to severe deep-seated disease that is frequently fatal. The *Candida albicans* commonly exist as commensal on mucocutaneous tissues of humans but can turn opportunistic when immunity is compromised. Our immune system deploys both innate and acquired reactions to curtail the candida excesses. Also, candida devise processes to evade immune responses on virtually all levels. Strikingly, whenever polymorphonuclear neutrophils (PMN) count is below moderate neutropenia, from cancer, chemotherapy, or stem cell transplant, then invasive candidiasis rears its head. The PMNs produced by the human body at copious amounts reaching a trillion cell per day in response to inflammatory demand. The PMNs react to pathogenic candida through phagocytosis, secretion of antimicrobial peptides, degradative enzymes, mineral chelators, and formation of neutrophils extracellular traps (NETs). Also, PMNs produce chemokines and cytokines to aid cross-talk with other immune cells and control their appropriate tropism to an area of need. Cell to cell contact apart from autocrine and paracrine communication of cell mediators are all put to use by PMNs. The PMNs carry a payload of a diverse group of lethal chemicals in specialized granules: azurophilic, specific, tertiary, and secretory vesicles. Intracellular signalling regulates the secretion of PMNs granules. Of note, the production of NETs provides PMN with extra ability to restrain pathogens in the body. Subpopulations of PMNs exist in different tissues carrying specialized functions.

Understanding the various roles of PMN in candidiasis can aid in the development of immunotherapy as the current antifungal therapy recorded a colossal setback.

**Keywords:** *Candida albicans*, neutrophils, granules, neutrophils extracellular traps (NETs).

**Introduction**

Candidiasis encompasses a spectrum of infection that ranges from superficial mucosal and cutaneous to systemic infection that involves internal organs. Invasive candidiasis causes morbidity and mortality globally among humans across all age groups (Tong and Tang, 2017). Some researchers reported a mortality figure above 70% (Pappas *et al.*, 2018). More than a dozen species of candida, cause disease in humans: *C. albicans* being the famous (Pappas *et al.*, 2018). Candida isolated in three-fifth of healthy humans frequently reside on the skin and mucous membrane: mainly gastrointestinal and urogenital tracts, which they often colonize (Nett, 2018; Pappas *et al.*, 2018). Deep-seated candidiasis may result from candidaemia from the primary colonizing site, but a non-hematogenous spread does occur (Clancy and Nguyen, 2018).

The immune system and microbiota control the commensal symbiosis against escalation to opportunistic infection (da Silva Dantas *et al.*, 2016). Immunosuppression, antibiotics, defect in host defenses, and advances in medical technology form a significant part of the predisposing factors to candidiasis (Singh *et al.*,

2015). Candidal bloodstream infections (BSIs) occur globally, with the most significant proportion from developing countries. The BSI has a burden of about 400,000 cases annually, with 3–5 per 100,000 incidences among the general population and 1–2% of ICU patients (Pappas *et al.*, 2018; Chowdhary *et al.*, 2017).

The diagnosis of invasive candidiasis poses a bottleneck in the management. Culture media is the gold standard, but only positive in less than half of deep-seated candidiasis, with associated reduced sensitivity and delayed outcome. Other non-culture methods are attracting researchers in recent times, but are short of antifungal sensitivity provided by culture media (Clancy and Nguyen, 2018; Nett, 2018). Treatment of invasive candidiasis with conventional azoles, amphotericin B, and newer generation echinocandins depicts increased resistance globally. Of note, the emergence of a nosocomial pathogen, *Candida auris* that is resistant to traditional therapy, is alarming (Chowdhary *et al.*, 2017).

### Immunity to *Candida*

A continuous layer of skin and mucous membranes keeps candida in check in the human host. While, infection usually results from a physiological and immunological imbalance that allows candidal overgrowth, onward interaction of epithelial cells and subsequent invasion. Nonetheless, epithelial cells discriminate between commensals and pathogens (Richardson *et al.*, 2018; Naglik *et al.*, 2017).

As reviewed by Brown and Colleagues, immune cells use both secretory and membrane-bound pathogen recognition receptors (PRRs) to recognize specific pathogen-associated molecular patterns (PAMPs) for the onward deployment of an appropriate immune response (Qin *et al.*, 2016; Brown, 2011). Phagocytes constitute a formidable defense to candidal pathogens (Brown, 2011). Neutrophils are vital cells to candidal immunity through phagocytosis, production of antimicrobial peptides, signal molecules, and extracellular traps (Amulic *et al.*, 2012).

The human body uses both innate and adaptive

immunity to counteract candidal infections (Netea *et al.*, 2015). The adaptive immune response plays a significant role in candidiasis, especially the cell-mediated immune response (Richardson and Moyes, 2015). Briefly, DCs activate helper T cells after recognition, phagocytosis, processing, and presentation of candidal antigen to CD4<sup>+</sup> T cells. The activated T cells undergo a clonal expansion of specific effector T cell subset aimed at curtailing the infection (Kashem *et al.*, 2015).

In Candidiasis, Th1 response results in protective immunity through IFN $\gamma$ , while deleterious outcomes result from Th2 immune response being anti-inflammatory (Deorukhkar, 2017). On mucocutaneous candidiasis, Th17 response protects human host, but controversy-laden its role in systemic infections (Bacher *et al.*, 2019). The IL-17 aids neutrophils recruitment to infection sites (Hernández-Santos and Gaffen, 2012). Impaired cell-mediated immunity predisposes the host to mucosal candidiasis, while neutropenia and defective innate barriers prompt hosts to disseminated candidiasis (Shoham and Levitz, 2005).

The role of humoral immunity for candidiasis in human and murine studies did not yield conclusive results (Fidel, 2002). Candidiasis lack specific antibodies, let alone their deficiency that predisposes to candidiasis, implying a non-protective role (Romani, 2004). Nevertheless, over time studies have documented antibody functions in anti-candidal immunity through opsonization, antibody-dependent cellular cytotoxicity, prevention of candidal adherence to epithelial surfaces and endothelial cells, and to a lesser extent neutralization. Of note, researchers found protective monoclonal antibodies for possible immunotherapy (Naglik, 2014).

The deficiency of neutrophils results in severe invasive candidiasis (Fulurija *et al.*, 1996, Fidel, 2002). Neutrophils were the most abundant leucocytes with the phagocytic ability and high turnover. Neutrophils are critical immune cells in candidiasis. Scientists reported an extended survival for neutrophils (Pillay *et al.*, 2010), also express MHC class II necessary for antigen

presentation (Fanger *et al.*, 1997). Evidence exists for neutrophils trafficking to peripheral lymph nodes (Beauvillain *et al.*, 2010). The above-enumerated properties imply the ability of neutrophils to activate T cells.

### **Polymorphonuclear Neutrophils (PMN) Biology**

Neutrophils, also called polymorphonuclear neutrophils (PMNs), are spherical with a diameter of 10 to 15  $\mu\text{m}$ . The PMNs are highly motile immune cells, have multiple cell surface projections: pseudopods to aid amoeboid locomotion in circulation and diapedesis across the endothelium. The PMNs possess multi-segmented nuclei: three to five lobes per cell in contrast to the closely associated eosinophils that have two lobes (Rosales, 2018, Amulic *et al.*, 2012). The PMNs represent the first group of immune cells recruited to the site of inflammation in huge numbers (Rosales, 2018).

The PMNs can recruit and activate other lymphocytes, dendritic cells, and monocyte through secreted chemokines and cytokines or direct contact. Of note, secreted cytokines from lymphocytes, monocytes, and macrophages can activate PMNs (Nathan, 2006). The PMNs have an immediate modulatory effect on B cells through the production of BAFF and APRIL. T cells through IFN- $\gamma$ , TNF- $\alpha$ , and GM-CSF potentiate the effector function of PMNs, but in general, PMNs have suppressive action towards T cells. The literature revealed a contradicting outcome on the cross-talk between  $\gamma\delta$  T cells and PMNs (Scapini and Cassatella, 2014).

The PMNs demonstrate an ability to process, express, and convey an antigen to peripheral lymph nodes T cell zone (Vono *et al.*, 2017). Several researchers postulate that neutrophils express MHC class II molecules (Beauvillain *et al.*, 2010; Oakes *et al.*, 2009; Abadie, 2005; Gosselin *et al.*, 1993). Therefore, the critical question here -does PMNs present *C. albicans* to T cells? If they do what effector function, they stimulate.

Another query here is, why neutrophil? - Neutrophils are motile, phagocytic, and secrete several chemokines, cytokines, degradative

enzymes, and toxic chemicals that are antimicrobial. However, does neutrophil live long enough, express MHC II molecules and tropic to lymph nodes: a sophistication needed for induction of specific CD4+ T cell-specific subset response? Furthermore, what happens to the body in terms of candidal infection in the absence of neutrophils? Figure 1 below summarizes the many functions of neutrophils.

As reviewed by Fidel and co-workers, neutrophils are indispensable in defense against systemic invasion by *C. albicans*. Moreover, cell-mediated immunity coordinated by CD4+ T cells is a prerequisite to protection against mucocutaneous candidiasis (Fidel, 2002). In essence, neutrophils have a secondary role of activating CD4+ T cells towards building cell-mediated immunity to candida, which implies bridging innate and adaptive immunity for effective control of candidiasis. Neutrophils clear invading pathogens principally through: phagocytoses of the opsonized particles, the release of antimicrobial molecules, and the release of chemokines (Nathan, 2006, Shen *et al.*, 1987).

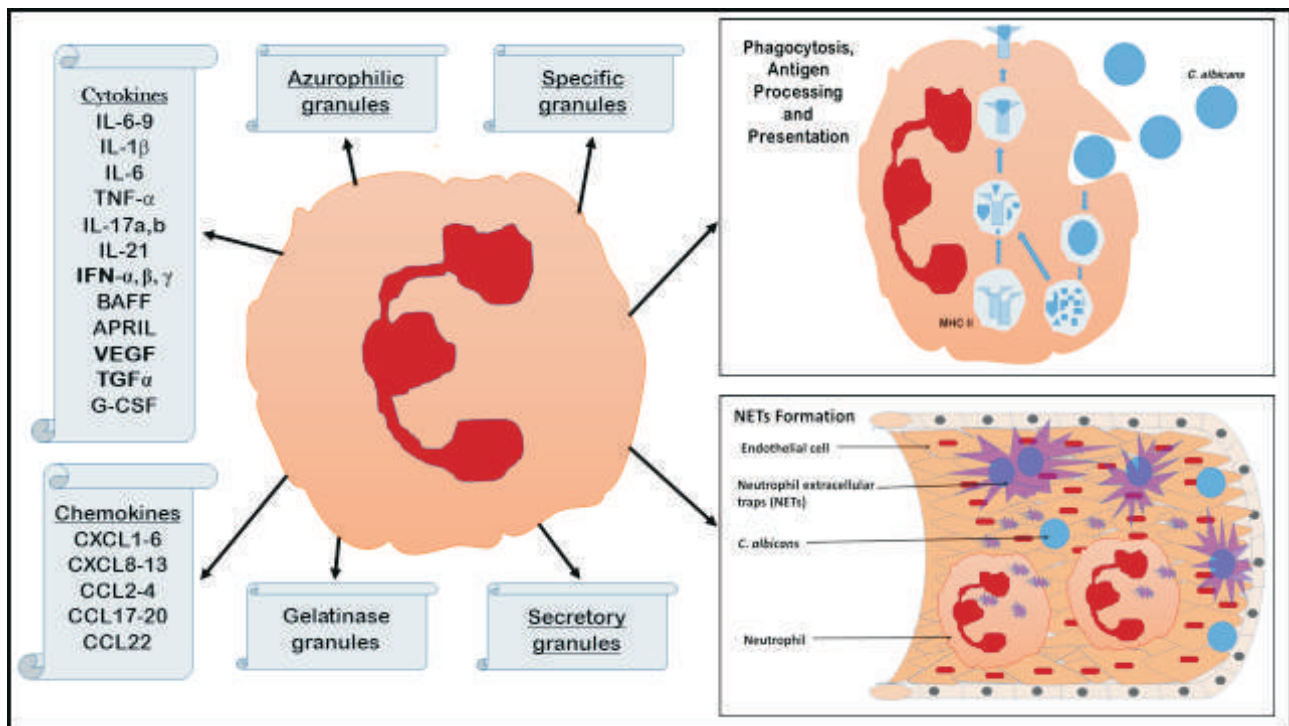
Tenth of billions of PMNs is produced daily with a life span of about six hours (Summers *et al.*, 2010), representing 40 to 60% of white blood cells (Chernecky and Berger, 2013). The PMNs normal count ranges between 1800 to 7700 cells/ $\mu\text{L}$  and a mean count of 4400 cells/ $\mu\text{L}$ . The PMNs count less than 1,700 cells/ $\mu\text{L}$  defined neutropenia, while PMNs count <500 cells/ $\mu\text{L}$  defined severe: absolute neutropenia. Often, individuals with mild neutropenia (PMNs count of 1,000 to 1,500 cells/ $\mu\text{L}$ ) are not prone to infection (Hébert *et al.*, 2018). The PMNs promptly migrate to the site of inflammation and wound healing (de Oliveira *et al.*, 2016). Still, if the PMNs failed recruitment, they mainly undergo programmed cell death and dispose of by macrophages through phagocytosis (Iba *et al.*, 2013).

The PMNs clear immune complexes and produce neutrophils extracellular traps (NETs) (Granger *et al.*, 2019). It is quite exciting how neutrophils produce extracellular DNA materials and secrete it on pathogens to restrict their

mobility and spread to adjacent and distant tissues. These cobweb-like products of neutrophils azurophilic granules cover pathogens and bind them to the endothelial walls in circulation. This process incapacitates pathogens *in vivo* through immobilization (Brinkmann, 2004).

The formation of PMN's net termed NETosis, happened in response to damaged-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs).

Generation of reactive oxygen species (ROS) initiates NETosis through activation of ERK and, ultimately, the myeloperoxidase (MPO) pathway. The MPO activates neutrophil elastase, which degrades actin and blocks phagocytosis. Henceforth, neutrophil elastase translocate to the nucleus to initiate degradation of chromatin that form NETs (Papayannopoulos, 2018). This phenomenon is critical in curtailing candidaemia, both hyphal and yeast form of candida, signifying yet another vital role of PMNs in *C. albicans* immunity.



**Figure 1: The many functions of neutrophils**

Researchers extensively documented neutrophil's significance as mediators of innate immunity. But due to their short life span, considered less than one day in the past, scientists perceive them not worthy sophisticated for more elaborate effector functions. In an *in vivo* labelling experiment, human neutrophils recorded a life span of 5.4 days in contrast to their perceived six hours of survival time (Pillay *et al.*, 2010). It is now apparent; the PMN lives long enough to be regarded as capable of elaborate effector functions coupled with their abilities mentioned above.

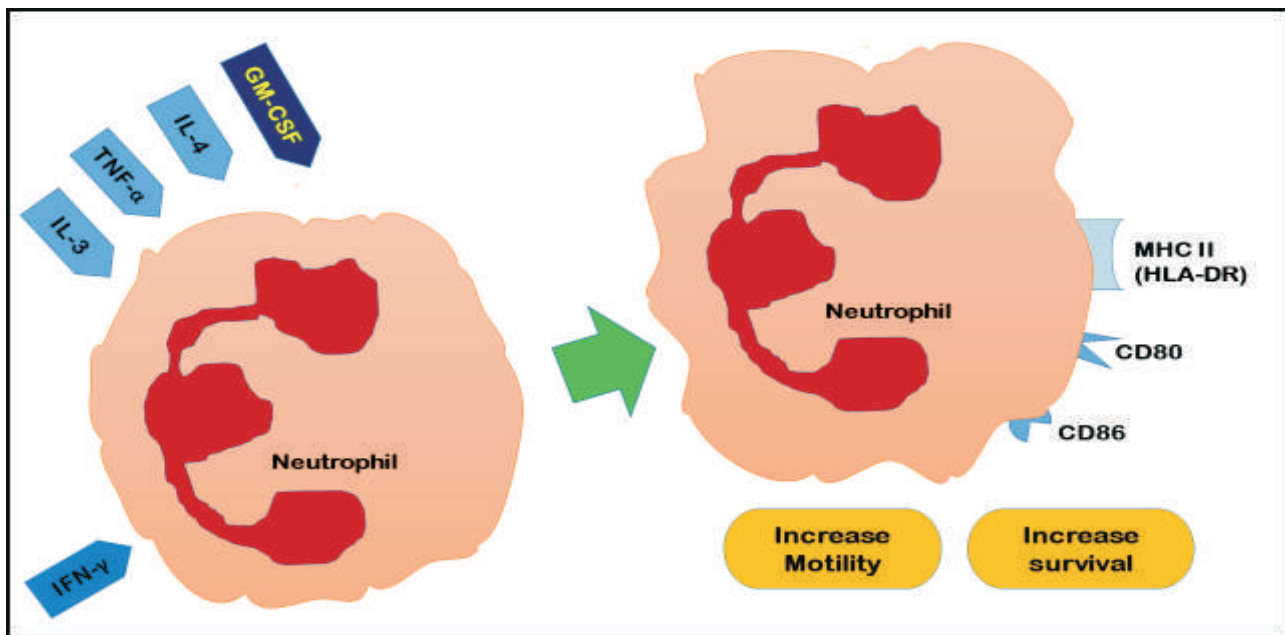
The PMNs, within the first day of their recruitment to the inflammatory site, they lose CD16 receptors from their cell surfaces. Followed by a decline in their phagocytic activity, and then they ultimately undergo apoptosis (Mortaz *et al.*, 2018; Perobelli *et al.*, 2015; Savill, 1997; Dransfield *et al.*, 1994). However, when exposed to cytokines, especially GM-CSF with or without IFN $\gamma$ , they preserve the CD16 live longer with maintained phagocytic activity (Perobelli *et al.*, 2015).

**The PMNs as Antigen Presenting Cells**

Experimentally, the spectrum of MHC class II expression by neutrophils from the peripheral

blood of healthy donors occurs as a bystander cell in an assay of the traditional antigen presentation by monocyte to CD4+ T cells. The spectrum of MHC class II expression by neutrophils occurred when neutrophils were cultured in the presence of GM-CSF, IFN $\gamma$ , and IL-3 (Gosselin *et al.*, 1993). In the same study, GM-CSF levelled the most salient among the three, and its MHC induction is dose-dependent.

IFN $\gamma$  and GM-CSF work in synergy, possessing a superior induction than any single one alone (Gosselin *et al.*, 1993, Lei *et al.*, 2001). Neutrophils cultured with GM-CSF, IFN $\gamma$ , and IL-4 revealed a similar result (Geng *et al.*, 2013). Figure 2 below depicts the schematic presentation of neutrophils priming with GM-CSF, IFN $\gamma$  with one of TNF- $\alpha$ , IL-3, or IL-4 to differentiate into an APC.

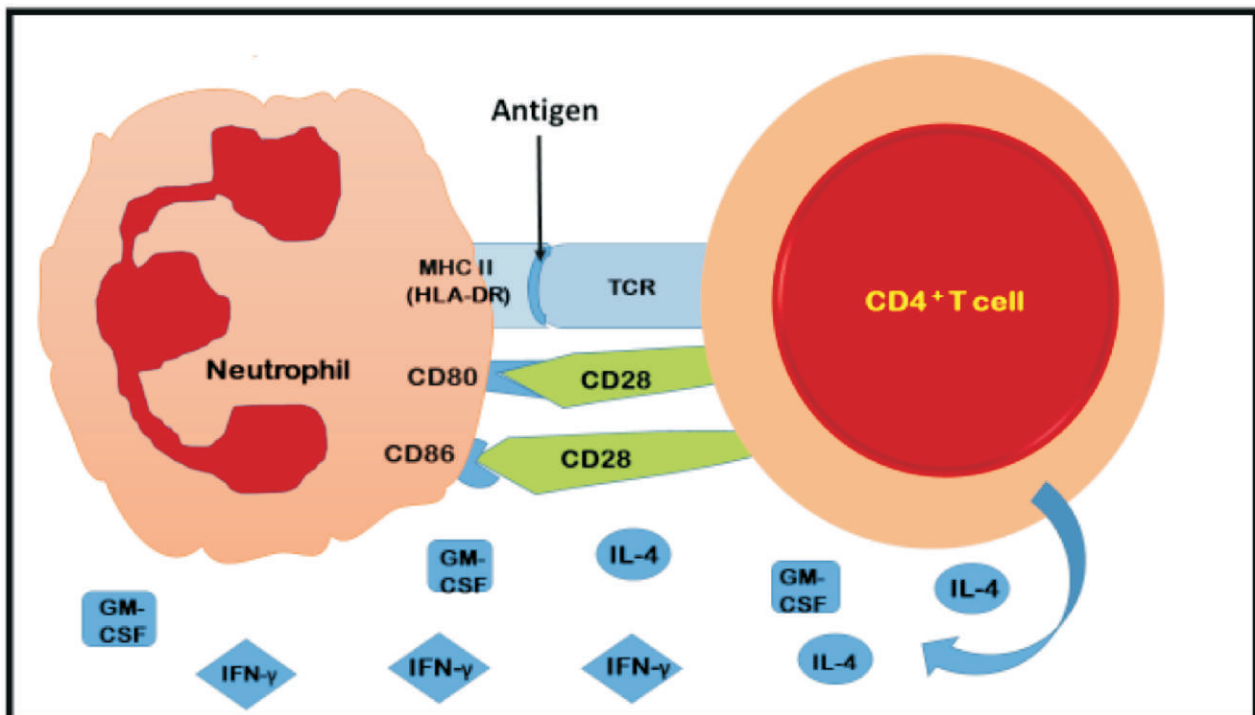


**Figure 2: Neutrophil priming for an antigen-presentation**

The MHC class II expression by neutrophils utilize a different pathway in presenting peptide antigen to CD4+ T cells. The alternate path becomes evident when primed neutrophils with the expression of MHC class II failed to activate CD4+ T cell response in the presence of tetanus toxoid (TT), just like the unprimed PMNs. It may be, PMNs can only present the antigen they engulf and process (Fanger *et al.*, 1997). The PMNs mediate superantigen presentation to CD4+ T cells both in vivo and in vitro when initially primed to produce MHC class II molecules by exposure to GM-CSF and IFN $\gamma$  (Radsak *et al.*, 2000; Fanger *et al.*, 1997). The aberration noticed here calls for further research to uncover this new pathway and possible utilization in designing immunotherapeutic targets.

with expression of MHC class II molecules on neutrophils when induced with GM-CSF and IFN $\gamma$ . The same explanation goes to the synergistic induction caused by this growth factor/cytokine on PMNs, meaning the same isotype: "HLA-DR" is a prerequisite to this synergy and not the other isotypes (Gosselin *et al.*, 1993). Human blood cells study reported subcellular compartmentation of CD80, CD86, and MHC II molecules in activated neutrophils that translocate to the cell surface cross-linking with CD11b (Sandilands *et al.*, 2005). Human neutrophils present antigens in MHC II (HLA-DR) dependent manner, but upregulation of the HLA-DR molecules require activated antigen-specific T cell (Vono *et al.*, 2017). Figure 3 below illustrates the description, as mentioned above, of PMNs antigen presentation capabilities.

An isotype of human leucocytes antigen (HLA) HLA-DR and not HLA-Dp and HLA-DQ linked



**Figure 3: Cross-talk between neutrophils as an APC with antigen-specific CD4<sup>+</sup> T cell**

The above crucial findings provide researchers with a basis for culturing neutrophils for five consecutive days using GM-CSF to maintain their viability. Antigen presentation by PMN to CD4<sup>+</sup> T cells means neutrophils are capable of bridging the innate to adaptive immunity. As such, activates cell-mediated immunity through T cells, which is a more specific, robust, qualitative, and sustained defense against *C. albicans* with an established memory of the oncoming exposure.

### Neutrophils Tropism

Neutrophils are highly motile cells utilizing their extracellular projections termed pseudopodia to get to the destination of inflammation. At their destination, they undergo a series of adhesion and transmigration along chemotactic molecules gradient to traverse the endothelium and extracellular matrix until they make contact with the area of infection or injury. At the inflammatory site, they participate actively in the immune response to the provoking agent primarily through phagocytosis (Oakes *et al.*, 2009). The PMNs interact with other immune cells through contact and secreted mediators at the site of inflammation and lymph node to orchestrate immune responses (Leliefeld *et al.*, 2015).

Human PMNs express a chemokine receptor CCR7 and migrate to lymph nodes in response to CCL19 and CCL21 ligands; dendritic cells and T cells migrate in the same way (Beauvillain *et al.*, 2010). Aged PMNs express CXCR4 that allows them to troop back to the bone marrow, the same receptor express maintain bone marrow neutrophils reserve (De Filippo and Rankin, 2018).

Compelling evidence exists that PMNs that receive survival signal *in vivo* can carry candida antigens to draining lymph nodes. It implies that conditioned PMNs transit into B or T cell zones of the lymph nodes, thereby initiating acquired immune response (Abadie, 2005). Different phenotypes of PMNs inhabit different organs (Rosales, 2018). Nevertheless, PMNs at the spleen marginal zone (MZ) secrete BAFF, APRIL, and IL-21 that promote somatic hypermutation and antibody production in B cells located in spleen's MZ (Puga *et al.*, 2012).

### Chemokines and Cytokines Associated with Neutrophils

For neutrophils to function as immune effector cells, they must possess receptors for chemokines and secrete cytokines too. In this regard, Altsteadt and his Colleagues

demonstrated that IL-8 is the principal cytokine secreted by non-stimulated neutrophils (Altstaedt *et al.*, 1996). As reviewed by Scapini and Cassatella (2014), PMNs can potentially secrete chemokines (C-X-C and C-C), cytokines (pro-inflammatory, anti-inflammatory, and immunomodulatory), angiogenic and fibrogenic factors, and colony-stimulating factors. The PMNs intracellular cytokine localization is still at large (Naegelen *et al.*, 2015).

The IL-8: monocyte-derived neutrophil chemotactic factor (MDNCF) coded by the IL 8 gene located on 4q12-21 of the human gene (Modi *et al.*, 1990). It has CXCR1 and CXCR2 as its chemokine receptors (Bacon *et al.*, 2002). This pro-inflammatory cytokine, IL-8, is secreted by other cells like epithelial cells, endothelial cells, macrophages, among others, apart from neutrophils. Its sole function concerning neutrophils includes chemotaxis, maturation, and recruitment from the bone marrow—also, rolling and diapedesis at the site of inflammation, and the processes involved in phagocytosis. Strikingly, IL-8 acts in an autocrine fashion on neutrophils (Altstaedt *et al.*, 1996). However, if stimulated neutrophils apart from IL-8 were capable of producing other cytokines like IL-1 $\beta$ , IL-6, and TNF- $\alpha$  (Zhang *et al.*, 2018).

Researchers established that neutrophils do express IL-17a in invasive fungal disease in dectin-1 and IL-23 dependant manner (Werner *et al.*, 2011). The IL-17a is a potent pro-inflammatory cytokine traditionally produce by Th17 cells, induced by IL-23. Studies incriminate the duo of IL 23 and IL-17 behind organ-specific autoimmunity in experimental autoimmune encephalitis (EAE) and inflammatory bowel disease (IBD), to mention but a few. It also plays a central role in neutrophils recruitment (Cua and Tato, 2010).

The IL-17a is crucial in immunity against invasive candidiasis, also links myeloid and lymphoid effector cells. In a murine study, IL-17AR double knockout shows decrease mice survival from invasive candidiasis and impaired neutrophils recruitment. Albeit, wild type contains the organism and demonstrated effective neutrophil recruitment to the site of

fungal inflammation (Huang *et al.*, 2004).

TNF- $\alpha$  is another salient cytokine secreted by neutrophils and by many cells in the human body. TNF- $\alpha$  is a pro-inflammatory cytokine implicated in the pathogenesis of autoimmune diseases like rheumatoid arthritis (RA) (Mitoma *et al.*, 2018, Végh *et al.*, 2020). Anti-TNF- $\alpha$  prone people to a high incidence of invasive fungal disease (Tragiannidis *et al.*, 2017; Styczyński, 2016).

Nevertheless, Aikawa and Colleagues (2016) found a lower prevalence of 0.5% vulvovaginal candidiasis in patients with rheumatoid arthritis taking TNF- $\alpha$  blockers, whereas no systemic candidiasis among the study groups. The role of TNF- $\alpha$  in defense against fungal pathogens is indisputable (Filler *et al.*, 2005), although excessive expression of TNF- $\alpha$  in inflammatory disease is detrimental (Rocha *et al.*, 2017).  
Granules of the PMNs

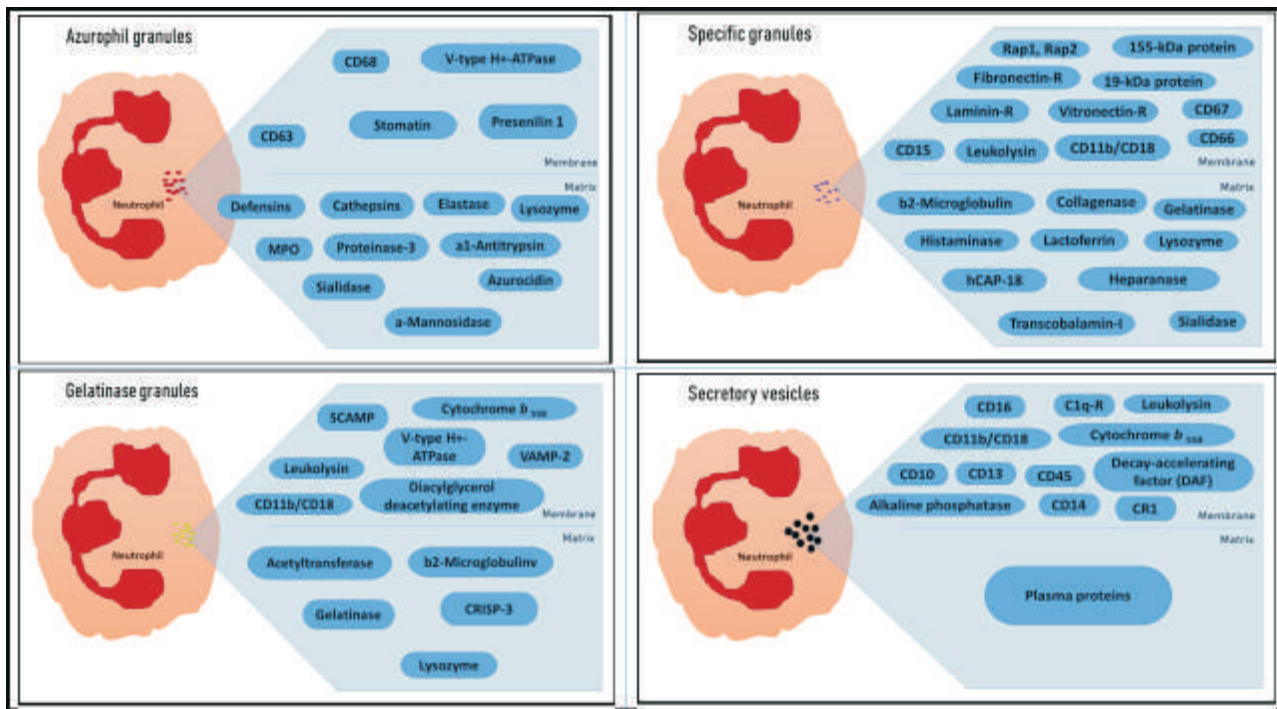
The PMNs harbour consignment of lethal armoury parked distinctively in three types of packages named based on their time of formation during PMNs development. They are primary (azurophilic), secondary (specific), and tertiary granules (gelatinase) (Nathan, 2006). The azurophilic granules contain myeloperoxidase (MPO). Specific granules contain abundant lactoferrin, while tertiary granules contain an enormous amount of gelatinase. The specific and tertiary granules lack MPO (Bedouhene *et al.*, 2020).

The PMNs produce secretory vesicles in addition to cytoplasmic granules as the fourth category. These payloads (granules and vesicles) contain antimicrobial peptides, proteases, respiratory burst oxidase, and various membrane-bound receptors (Faurischou and Borregaard, 2003). Neutrophils: viewed as death packages. Figure 4 below summarizes the chemical constituents of the PMNs four main types of packages.

The PMN's granules are vital to its effector innate infantry role. The secondary and tertiary granules are abundant in Ls and Ms: lactoferrin, lipocalin, lysozyme, and LL37, while MMP8,

MMP9, and MMP25 complete the list (Nathan, 2006). The azurophilic granules contain serine proteases, proteinases, cathepsin G, elastase, CAP37, NSP4, HNPs, and bacterial permeability-increasing protein (BPI) in addition to MPO (Cowland and Borregaard, 2016).

The secretory vesicles are discovered much later, released earlier in inflammation, and richly contains adhesion molecules (Masgrau-Alsina *et al.*, 2020). Researchers unfolded several intracellular signalling molecules that tightly coordinate the formation, intracellular movement, and discharge of PMN's granules and secretory vesicles (Lacy, 2006).



**Figure 4: The constituent of PMN's Granules**

### Host versus Candida in the Absence of Neutrophils

Experimental animals treated with neutrophil specific monoclonal antibody to aid neutrophil depletion (neutropenia) developed both local and invasive candidaemia, which is rapidly progressive upon exposure to the pathogen. In this situation, a higher burden of candida at tissue level with disastrous pathology results (Fulurija *et al.*, 1996). So, for the experimental mice to develop progressively fatal infection secondary to depletion of only neutrophils implies neutrophils are playing a critical role for candidal pathogens immunity.

Scientist reported in 1966 at an oncology unit, a significantly higher incidence of invasive candidiasis among leukaemic patients on corticosteroids. These groups of patients characteristically bear neutropenia (Bodey,

1966). The outcome further highlighted the role of neutrophils in immunity against candida pathogens.

Patients with haematological cancers and neutropenia frequently encounter invasive candidiasis that recorded higher mortality (Chandrasekar *et al.*, 2018). Often, haematopoietic stem cell transplant recipients with neutropenia encounter invasive candidiasis too; invasive fungal infection leads the list of mortality cause among these patients (Omrani and Almaghrabi, 2017; Kontoyiannis *et al.*, 2010).

Patients on stem cell therapy or chemotherapy that experience prolong neutropenia are especially prone to deep-seated candidiasis (van Prehn *et al.*, 2017). Researchers found acute leukaemic patients on inductive therapy to harbour neutropenia commonly complicated by invasive candidiasis



(Guarana and Nucci, 2018). Hematologic patients with neutropenia regularly experienced fatal systemic candidiasis, but patients' survival significantly improved with the resolution of neutropenia (Gamaletsou *et al.*, 2014).

### Conclusion

As discussed previously, innate immune response plays a principal role in *C. albicans* infection ranging from barrier to prevention of systemic disease, where neutrophil proves a promising effector cell. As we discussed in this review, neutropenia from multiple causes results in invasive candidiasis signifying the undisputable role of PMNs in gatekeeping against candidal infection. We reviewed the various effector mechanisms employ by PMN to curtail candidiasis with the hope that future researches will look deeper into these mechanisms to come up with specific targeted therapy (immunotherapy) to candidiasis.

### Acknowledgment

Not applicable

### Conflict of Interests

The authors declare no conflict of interest.

### Funding/Support

No funding or support available to this paper.

### References

Abadie, V. 2005. Neutrophils rapidly migrate via lymphatics after Mycobacterium bovis BCG intradermal vaccination and shuttle live bacilli to the draining lymph nodes. *Blood*; 106, 1843-1850.

Aikawa, N.E., Rosa, D.T., Del Negro, G.M., Moraes, J.C., Ribeiro, A.C., Saad, C.G., Silva, C. A. and Bonfá, E. (2016). Systemic and localized infection by *Candida* species in patients with rheumatic diseases receiving anti-TNF therapy. *Revista Brasileira de Reumatologia*; 56: 478-482.

Altstaedt, J., Kirchner, H. and Rink, L. (1996). Cytokine production of neutrophils is limited to interleukin-8. *Immunology*; 89: 563-568.

Amulic, B., Cazalet, C., Hayes, G.L., Metzler, K.D. and Zychlinsky, A. (2012). Neutrophil function: from mechanisms to disease. *Annual Review in Immunology*; 30: 459-89.

Bacher, P., Hohnstein, T., Beerbaum, E., Röcker, M., Blango, M. G., Kaufmann, S., Röhmel, J., Eschenhagen, P., Grehn, C., Seidel, K., Rickerts, V., Lozza, L., Stervbo, U., Nienen, M., Babel, N., Milleck, J., Assenmacher, M., Cornely, O.A., Ziegler, M., Wisplinghoff, H., Heine, G., Worm, M., Siegmund, B., Maul, J., Creutz, P., Tabeling, C., Ruwwe-Glösenkamp, C., Sander, L. E., Knosalla, C., Brunke, S., Hube, B., Kniemeyer, O., Brakhage, A. A., Schwarz, C. and Scheffold, A. 2019. Human Anti-fungal Th17 Immunity and Pathology Rely on Cross-Reactivity against *Candida albicans*. *Cell*; 176: 1340-1355.e15.

Bacon, K., Baggiolini, M., Broxmeyer, H., Horuk, R., Lindley, I., Mantovani, A., Maysushima, K., Murphy, P., Nomiyama, H., Oppenheim, J., Rot, A., Schall, T., Tsang, M., Thorpe, R., Van Damme, J., Wadhwa, M., Yoshie, O., Zlotnik, A. and Zoon, K. (2002). Chemokine/chemokine receptor nomenclature. *Journal of Interferon Cytokine Research*; 22: 1067-1068.

Beauvillain, C., Cunin, P., Doni, A., Scotet, M., Jaillon, S., Loiry, M. L., Magistrelli, G., Masternak, K., Chevailler, A., Delneste, Y. and Jeannin, P. (2010). CCR7 is involved in the migration of neutrophils to lymph nodes. *Blood*; 117: 1196-1204.

Bedouhene, S., Dang, P. M.-C., Hurtado-Nedelec, M. & El-Benna, J. (2020). Neutrophil Degranulation of Azurophil and Specific Granules. *Methods in Molecular Biology*; 2087: 215-222.

Bodey, G. P. (1966). Fungal infections complicating acute leukemia. *Journal of Chronic Diseases*; 19: 667-687.

Brinkmann, V. (2004). Neutrophil Extracellular Traps Kill Bacteria. *Science*; 303: 1532-1535.

Brown, G.D. (2011). Innate Antifungal Immunity: The Key Role of Phagocytes. *Annual Review of Immunology*; 29: 1-21.

Chandrasekar, P., Sirohi, B., Seibel, N. L., Hsu, J. W., Azie, N., Wu, C. and Ruhnke, M. (2018). Efficacy of micafungin for the treatment of invasive candidiasis and candidaemia in patients with neutropenia. *Mycoses*; 61: 331-336.

Chernecky, C. and Berger, B. (2013). Differential leukocyte count (diff)-peripheral blood. *Laboratory Tests and*

- Diagnostic Procedures: 440-446.
- Chowdhary, A., Sharma, C. and Meis, J. F. (2017). *Candida auris*: A rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. *PLOS Pathogens*;13: e1006290.
- Clancy, C. J. and Nguyen, M. H. (2018). Diagnosing Invasive Candidiasis. *Journal of Clinical Microbiology*: 56.
- Cowland, J. B. & Borregaard, N. (2016). Granulopoiesis and granules of human neutrophils. *Immunology Review*; 273: 11-28.
- Cua, D. J. and Tato, C. M. 2010. Innate IL-17-producing cells: the sentinels of the immune system. *Nature Reviews Immunology*; 10: 479-489.
- Da Silva Dantas, A., Lee, K. K., Raziunaite, I., Schaefer, K., Wagener, J., Yadav, B. and Gow, N. A. (2016). Cell biology of *Candida albicans*–host interactions. *Current Opinion in Microbiology*; 34: 111-118.
- De Filippo, K. & Rankin, S. M. (2018). CXCR4, the master regulator of neutrophil trafficking in homeostasis and disease. *European Journal of Clinical Investigation*; 48(2): e12949-e12949.
- De Oliveira, S., Rosowski, E.E. and Huttenlocher, A. (2016). Neutrophil migration in infection and wound repair: going forward in reverse. *Nature reviews. Immunology*; 16: 378-391.
- Deorukhkar, S. (2017). Immunity to *Candida* infection: an overview. *J MOJ Immunol*, 5, 00144.
- Dransfield, I., Buckle, A., Savill, J. S., McDowall, A., Haslett, C. and Hogg, N. 1994. Neutrophil apoptosis is associated with a reduction in CD16 (Fc gamma RIII) expression. *The Journal of Immunology*; 153: 1254-1263.
- Fanger, N. A., Liu, C., Guyre, P. M., Wardwell, K., O'neil, J., Guo, T. L., Christian, T. P., Mudzinski, S. P. and Gosselin, E.J. (1997). Activation of human T cells by major histocompatibility complex class II expressing neutrophils: proliferation in the presence of superantigen, but not tetanus toxoid. *Blood*; 89: 4128-4135.
- Faurschou, M. and Borregaard, N. 2003. Neutrophil granules and secretory vesicles in inflammation. *Microbes and Infections*; 5: 1317-1327.
- Fidel, P. L. (2002). Immunity to *Candida*. *Oral Diseases*, 8, 69-75.
- Filler, S. G., Yeaman, M. R. and Sheppard, D. C. (2005). Tumor Necrosis Factor Inhibition and Invasive Fungal Infections. *Clinical Infectious Diseases*; 41: S208-S212.
- Fulurija, A., Ashman, R. B. and Papadimitriou, J. M. (1996). Neutrophil depletion increases susceptibility to systemic and vaginal candidiasis in mice, and reveals differences between brain and kidney in mechanisms of host resistance. *Microbiology*; 142: 3487-3496.
- Gamaletsou, M. N., Walsh, T. J., Zaoutis, T., Pagoni, M., Kotsopoulou, M., Voulgarelis, M., Panayiotidis, P., Vassilakopoulos, T., Angelopoulou, M. K., Marangos, M., Spyridonidis, A., Kofteridis, D., Pouli, A., Sotiropoulos, D., Matsouka, P., Argyropoulou, A., Perloretzou, S., Leckerman, K., Manaka, A., Oikonomopoulos, P., Daikos, G., Petrikkos, G. & Sipsas, N. V. (2014). A prospective, cohort, multicentre study of candidaemia in hospitalized adult patients with haematological malignancies. *Clinical Microbiology and Infection*; 20: 050-057.
- Geng, S., Matsushima, H., Okamoto, T., Yao, Y., Lu, R. and Takashima, A. (2013). Reciprocal regulation of development of neutrophil-dendritic cell hybrids in mice by IL-4 and interferon-gamma. *PLoS One*; 8: e82929.
- Gosselin, E., Wardwell, K., Rigby, W. And Guyre, P. (1993). Induction of MHC class II on human polymorphonuclear neutrophils by granulocyte/macrophage colony-stimulating factor, IFN-gamma, and IL-3. *The Journal of Immunology*; 151: 1482-1490.
- Granger, V., Peyneau, M., Chollet-Martin, S. and De Chaisemartin, L. (2019). Neutrophil Extracellular Traps in Autoimmunity and Allergy: Immune Complexes at Work. *Frontiers in Immunology*; 10: 2824.
- Guarana, M. and Nucci, M. (2018). Acute disseminated candidiasis with skin lesions: a systematic review. *Clinical Microbiology and Infection*; 24: 246-250.
- Hébert, L., Cimon, M., Ohayon, D., Loupis, N. and Piergallini, R. (2018). Compositions and methods for the treatment of mucositis associated with treatment-induced

- neutropenia. USA patent application US 2018/0256914A1.
- Hernández-Santos, N. and Gaffen, S. L. 2012. Th17 cells in immunity to *Candida albicans*. *Cell Host and Microbe*; 11: 425-435.
- Huang, W., Na, L., Fidel, Paul L. and Schwarzenberger, P. (2004). Requirement of Interleukin-17A for Systemic Anti-*Candida albicans* Host Defense in Mice. *The Journal of Infectious Diseases*; 190: 624-631.
- Iba, T., Hashiguchi, N., Nagaoka, I., Tabe, Y. and Murai, M. (2013). Neutrophil cell death in response to infection and its relation to coagulation. *Journal of Intensive Care*; 1: 13.
- Kashem, S.W., Igyarto, B.Z., Gerami-Nejad, M., Kumamoto, Y., Mohammed, J.A., Jarrett, E., Drummond, R.A., Zurawski, S. M., Zurawski, G., Berman, J., Iwasaki, A., Brown, G.D. and Kaplan, D.H. (2015). *Candida albicans* morphology and dendritic cell subsets determine T helper cell differentiation. *Immunity*; 42: 356-366.
- Kontoyiannis, D. P., Marr, K. A., Park, B. J., Alexander, B. D., Anaissie, E. J., Walsh, T. J., Ito, J., Andes, D. R., Baddley, J. W., Brown, J. M., Brumble, L. M., Freifeld, A. G., Hadley, S., Herwaldt, L. A., Kauffman, C. A., Knapp, K., Lyon, G. M., Morrison, V. A., Papanicolaou, G., Patterson, T. F., Perl, T. M., Schuster, M. G., Walker, R., Wannemuehler, K. A., Wingard, J. R., Chiller, T. M. and Pappas, P.G. (2010). Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clinical Infectious Diseases*; 50: 1091-100.
- Lacy, P. (2006). Mechanisms of Degranulation in Neutrophils. *Allergy, Asthma & Clinical Immunology*; 2: 98.
- Lei, L., Altstaedt, J., Von Der Ohe, M., Proft, T., Gross, U. and Rink, L. (2001). Induction of interleukin-8 in human neutrophils after MHC class II cross-linking with superantigens. *Journal of Leukocyte Biology*; 70: 80-86.
- Lelifeld, P. H., Koenderman, L. and Pillay, J. (2015). How neutrophils shape adaptive immune responses. *Journal Frontiers in Immunology*; 6: 471.
- Masgrau-Alsina, S., Sperandio, M. & Rohwedder, I. (2020). Neutrophil recruitment and intracellular vesicle transport: A short overview. *European Journal of Clinical Investigation*; 50: e13237.
- Mitoma, H., Horiuchi, T., Tsukamoto, H. and Ueda, N. (2018). Molecular mechanisms of action of anti-TNF- $\alpha$  agents—Comparison among therapeutic TNF- $\alpha$  antagonists. *Journal of Cytokine*; 101: 56-63.
- Modi, W., Dean, M., Seuanes, H., Mukaida, N., Matsushima, K. and O'Brien, S. (1990). Monocyte-derived neutrophil chemotactic factor (MDNCF/IL-8) resides in a gene cluster along with several other members of the platelet factor 4 gene superfamily. *Human Genetics*: 84.
- Mortaz, E., Alipoor, S. D., Adcock, I. M., Mumby, S. and Koenderman, L. (2018). Update on Neutrophil Function in Severe Inflammation. *Frontiers in Immunology*; 9: 2171-2171.
- Naegelen, I., Beaume, N., Plançon, S., Schenten, V., Tschirhart, E. J. and Bréchar, S. (2015). Regulation of Neutrophil Degranulation and Cytokine Secretion: A Novel Model Approach Based on Linear Fitting. *Journal of Immunology Research*; 2015: 817038.
- Naglik, J. R. (2014). *Candida* immunity. *New Journal of Science*: 390241.
- Naglik, J. R., König, A., Hube, B. and Gaffen, S. L. (2017). *Candida albicans*—epithelial interactions and induction of mucosal innate immunity. *Current Opinion in Microbiology*; 40: 104-112.
- Nathan, C. (2006). Neutrophils and immunity: challenges and opportunities. *Nature Reviews Immunology*; 6: 173-182.
- Netea, M.G., Joosten, L.A., Van Der Meer, J.W., Kullberg, B. J. and Van De Veerdonk, F.L. (2015). Immune defence against *Candida* fungal infections. *Nature Review of Immunology*; 15: 630-642.
- Nett, J.E. (2018). Special Issue: *Candida* and Candidiasis. *Journal of Fungi (Basel)*: 4.
- Oakes, P. W., Patel, D. C., Morin, N. A., Zitterbart, D. P., Fabry, B., Reichner, J. S. and Tang, J. X. (2009). Neutrophil morphology and migration are affected by substrate elasticity. *Blood*; 114: 1387-1395.

- Omrani, A.S. and Almaghrabi, R.S. 2017. Complications of hematopoietic stem transplantation: Fungal infections. *Hematological Oncology and Stem Cell Therapy*; 10: 239-244.
- Papayannopoulos, V. (2018). Neutrophil extracellular traps in immunity and disease. *Nature Reviews Immunology*; 18: 134-147.
- Pappas, P.G., Lionakis, M.S., Arendrup, M.C., Ostrosky-Zeichner, L. and Kullberg, B.J. (2018). Invasive candidiasis. *Nature Reviews Disease Primers*; 4: 18026.
- Perobelli, S. M., Galvani, R. G., Gonçalves-Silva, T., Xavier, C. R., Nóbrega, A. and Bonomo, A. (2015). Plasticity of neutrophils reveals modulatory capacity. *Journal Brazilian Journal of Medical and Biological Research*; 48: 665-675.
- Pillay, J., Den Braber, I., Vriskoop, N., Kwast, L. M., De Boer, R. J., Borghans, J. A. M., Tesselaar, K. and Koenderman, L. 2010. In vivo labeling with 2H<sub>2</sub>O reveals a human neutrophil lifespan of 5.4 days. *Blood*; 116: 625-627.
- Puga, I., Cols, M., Barra, C. M., He, B., Cassis, L., Gentile, M., Comerma, L., Chorny, A., Shan, M. and Xu, W. (2012). B cell-helper neutrophils stimulate the diversification and production of immunoglobulin in the marginal zone of the spleen. *Nature Immunology*, 13: 170-180.
- Qin, Y., Zhang, L., Xu, Z., Zhang, J., Jiang, Y.-Y., Cao, Y. and Yan, T. (2016). Innate immune cell response upon *Candida albicans* infection. *Virulence*; 7: 512-526.
- Radsak, M., Iking-Konert, C., Stegmaier, S., Andrassy, K. and Hansch, G. M. (2000). Polymorphonuclear neutrophils as accessory cells for T-cell activation: major histocompatibility complex class II restricted antigen-dependent induction of T-cell proliferation. *Immunology*; 101: 521-530.
- Richardson, J. P., Ho, J. and Naglik, J. R. (2018). *Candida*-Epithelial Interactions. *Journal of fungi (Basel, Switzerland)*; 4: 22.
- Richardson, J. P. and Moyes, D. L. (2015). Adaptive immune responses to *Candida albicans* infection. *Virulence*; 6: 327-337.
- Rocha, F.A.C., Alves, A.M.C.V., Rocha, M.F.G., De Aguiar Cordeiro, R., Brilhante, R. S. N., Pinto, A. C.M.D., De Melo Nunes, R., Girão, V.C.C. and Sidrim, J.J.C. (2017). Tumor necrosis factor prevents *Candida albicans* biofilm formation. *Journal of Scientific Reports*; 7: 1-7.
- Romani, L. (2004). Immunity to fungal infections. *Nature Reviews Immunology*, 4, 11-24.
- Rosales, C. 2018. Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types? *Frontiers of Physiology*; 9: 113.
- Sandilands, G.P., Ahmed, Z., Perry, N., Davison, M., Lupton, A. and Young, B. (2005). Cross-linking of neutrophil CD11b results in rapid cell surface expression of molecules required for antigen presentation and T-cell activation. *Immunology*; 114: 354-368.
- Savill, J. 1997. Apoptosis in resolution of inflammation. *Journal of Leukocyte Biology*; 61: 375-380.
- Scapini, P. and Cassatella, M.A. (2014). Social networking of human neutrophils within the immune system. *Blood*; 124: 710-9.
- Shen, L., Guyre, P. & Fanger, M. (1987). Polymorphonuclear leukocyte function triggered through the high affinity Fc receptor for monomeric IgG. *The Journal of Immunology*, 139: 534-538.
- Shoham, S. and Levitz, S. M. 2005. The immune response to fungal infections. *British Journal of Haematology*; 129: 569-582.
- Singh, S., Fatima, Z. & Hameed, S. (2015). Predisposing factors endorsing *Candida* infections. *Infection Medicine*; 23: 211-223.
- Styczyński, J. (2016). Invasive pulmonary aspergillosis in a child with acute myeloid leukaemia: pharmacotherapy and surgical management. *Journal of Oncology Review*; 6: A169-174.
- Summers, C., Rankin, S.M., Condliffe, A.M., Singh, N., Peters, A.M. and Chilvers, E.R. (2010). Neutrophil kinetics in health and disease. *Trends in Immunology*; 31: 318-324.
- Tong, Y. and Tang, J. (2017). *Candida albicans* infection and intestinal immunity. *Microbiological Research*; 198: 27-35.
- Tragiannidis, A., Kyriakidis, I., Zündorf, I. and Groll, A. H. 2017. Invasive fungal infections in pediatric patients treated with tumor necrosis alpha (TNF- $\alpha$ ) inhibitors. *Journal of Mycoses*; 60: 222-229.
- Van Prehn, J., Menke-Van Der Houven Van Oordt, C. W., De Rooij, M. L., Meijer, E.,

- Bomers, M. K. and Van Dijk, K. (2017). Hepatosplenic Candidiasis Without Prior Documented Candidemia: An Underrecognized Diagnosis? *Oncologist*; 22: 989-994.
- Végh, E., Kerekes, G., Pusztai, A., Hamar, A., Szamosi, S., Vánca, A., Bodoki, L., Pogácsás, L., Balázs, F. and Hodosi, K. (2020). Effects of 1-year anti-TNF- $\alpha$  therapy on vascular function in rheumatoid arthritis and ankylosing spondylitis. *Journal of Rheumatology International*; 40: 427-436.
- Vono, M., Lin, A., Norrby-Teglund, A., Koup, R. A., Liang, F. and Loré, K. 2017. Neutrophils acquire the capacity for antigen presentation to memory CD4(+) T cells in vitro and ex vivo. *Blood*; 129:1991-2001.
- Werner, J. L., Gessner, M. A., Lilly, L. M., Nelson, M. P., Metz, A. E., Horn, D., Dunaway, C. W., Deshane, J., Chaplin, D. D., Weaver, C. T., Brown, G. D. and Steele, C. 2011. Neutrophils Produce Interleukin 17A (IL-17A) in a Dectin-1- and IL-23-Dependent Manner during Invasive Fungal Infection. *Infection and Immunity*; 79: 3966-3977.
- Zhang, C., Shu, W., Zhou, G., Lin, J., Chu, F., Wu, H. and Liu, Z. (2018). Anti-TNF- $\alpha$  therapy suppresses pro-inflammatory activities of mucosal neutrophils in inflammatory bowel disease. *Journal of Mediators of Inflammation*; 2018: 3021863.

**Citation:** Aliyu, M., Muhammed, Y., Azeez-Akande, O. and Jibril, M. Neutrophils: The Gatekeepers to Candidal Infection. *Sokoto Journal of Medical Laboratory Science*; 5(3):17 - 29.

**Copyright.** This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.