The increasing use of broad-spectrum antibiotics and immunosuppressive therapies disrupts the natural bacterial microbiota, creating favorable conditions for fungal pathogen proliferation. Patients undergoing prolonged hospitalization and individuals with impaired immune function are particularly susceptible to such infections. In recent years, fungal infections have also emerged as a significant complication in patients with COVID-19.

The main causative agents of fungal infections are yeast species of the *Candida* genus, among which *Candida albicans* remains the most frequently isolated and well-characterized species. Although *C. albicans* is a natural component of the human microbiota, disturbances in host homeostasis can lead to excessive proliferation, resulting in infections ranging from mild superficial conditions to severe systemic diseases associated with high mortality rates. *C. albicans* exhibits remarkable adaptive capabilities and a wide array of virulence factors that enable survival in diverse host environments. One of the most effective survival strategies and a major challenge in treating *C. albicans* infections is biofilm formation.

Biofilms are multicellular structures that exhibit enhanced resistance to both host immune defenses and antifungal therapies. This resistance is largely attributed to the production of an extracellular matrix (ECM), which acts as a physical barrier composed of various polymeric compounds. ECM consists primarily of polysaccharides (particularly β-glucans and mannans), proteins, lipids, secondary metabolites, and extracellular nucleic acids (eDNA and eRNA). Among these components, nucleic acids remain the least studied, although growing evidence suggests they contribute to biofilm stability due to their physicochemical properties and also influence interactions with the host immune system.

Biofilms are not only protective structures, but also unique microenvironments characterized by gradients of oxygen, pH, and nutrients. A key research question remains how biofilm development, its ECM components, and the specific microenvironment modulate immune cell responses.

Neutrophils, the most abundant leukocyte population, play a central role in combating fungal infections, constituting the first line of host defense. These cells have a short lifespan and rely on rapid pathogen-neutralization mechanisms. In addition to classical strategies such as phagocytosis and degranulation, neutrophils employ a specific mechanism known as NETosis, which involves the release of neutrophil extracellular traps (NETs). The activity of neutrophils is supported by macrophages, which participate in all phases of the immune response.

An important mediator of innate immunity in fungal infections is the LL-37 peptide, produced primarily by neutrophils, as well as epithelial cells and keratinocytes. LL-37 exhibits

both antifungal and immunomodulatory properties. Notably, LL-37 can form complexes with negatively charged molecules such as DNA and RNA, potentially influencing immune response modulation.

Previous studies have demonstrated that *C. albicans* biofilm formation can significantly impact the host immune response, leading to its suppression or dysregulation. However, the underlying mechanisms of this phenomenon remain poorly characterized. The ECM appears to play a crucial role in this process, serving as the first point of contact between biofilms and immune cells while also affecting oxygen diffusion and shaping the local microenvironment.

In the first part of this study, the potential mechanism of NETosis activation in response to fungal nucleic acids was investigated, as these molecules constitute a significant component of the *C. albicans* ECM. Emphasis was placed on characterizing the signaling cascade leading to NADPH oxidase activation. The results demonstrated that *C. albicans* nucleic acids induce NETosis in a ROS-dependent manner through the activation of Syk, ERK1/2, p38 MAPK, PI3K, and PKC kinases, which collectively regulate NADPH oxidase activation. This cascade leads to increased reactive oxygen species (ROS) production, a key trigger for NETosis.

Considering the high concentration of LL-37 released at infection sites and its ability to form complexes with negatively charged molecules, the impact of LL-37 on neutrophil responses induced by fungal nucleic acids was examined. The findings indicate that LL-37 modifies neutrophil activity by suppressing ROS-dependent NETosis. Despite this suppression, a shift in response toward increased chemotaxis and overall immune mobilization was observed, as reflected by enhanced IL-8 release. These results suggest that LL-37 plays a key role in neutrophil response modulation by limiting the cytotoxic effects of excessive ROS and NET release while promoting the induction of longer-lasting proinflammatory mechanisms.

Given the crucial role of type I interferons (IFN-I) in nucleic acid-induced immune responses, the study was expanded to assess the effects of fungal nucleic acids and their complexes with LL-37 on IFN-β production in human THP-1-derived macrophages. The findings demonstrated that LL-37, through interactions with fungal DNA, enhances activation of the cytoplasmic cGAS-STING-IRF3 pathway by increasing the availability of *C. albicans* DNA for cGAS recognition, thereby amplifying IFN-β production. Additionally, fungal RNA alone strongly induced IFN-β production, independent of LL-37. Furthermore, LL-37 binding to both fungal DNA and RNA influenced macrophage polarization toward a proinflammatory phenotype, characterized by increased nitric oxide (NO) production and elevated CD86 expression. This macrophage response profile may play a significant role in shaping immune

regulation during fungal infections, affecting both pathogen clearance and potential pathological inflammatory processes.

The second part of this study aimed to analyze neutrophil responses within the biofilm microenvironment, considering not only individual virulence factors but also the physiological conditions present in biofilms. Particular attention was given to hypoxia, a key factor generated within the biofilm structure, which may play a crucial role in immune modulation during later stages of fungal infection.

The results showed that hypoxia, largely driven by the presence of a dense layer of mannans and glucans, promotes HIF-1α accumulation in neutrophils, leading to significant functional reprogramming. Under increased hypoxic conditions, NETosis and ROS production were suppressed, while neutrophil survival remained high, and oxygen-independent mechanisms such as IL-8 and MIP-1β production and degranulation were enhanced. Degranulation was particularly notable, as it was associated with increased neutrophil elastase activity in the biofilm environment. Neutrophil elastase, a proteolytic enzyme involved in pathogen clearance, was also found to contribute to tissue damage.

In the final stage of this research, the potential role of neutrophil elastase in promoting *C. albicans* epithelial invasion was explored. Fluorescence microscopy analyses revealed that neutrophil elastase binds to the fungal cell wall and may be exploited by *C. albicans* to enhance epithelial damage in lung cells (A549). These findings suggest that, in the biofilm context, where neutrophil degranulation becomes the dominant effector mechanism at later infection stages, the release of proteolytic enzymes may paradoxically facilitate fungal invasiveness rather than limit its spread.

The findings presented provide novel insights into the complex pathogen-host interactions, emphasizing the critical role of ECM components and the biofilm microenvironment in immune response modulation.