**The network interplay of type 1 interferon and toll-like receptor signaling cascades
hallmarks the immune response against *Candida spp.* infections**

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ABSTRACT

Here we employed a stepwise, integrative, and systems immunology approach to unravel the human immune responses to *C. albicans* and *C. auris* by analyzing publicly available human transcriptome data. Modular gene co-expression analysis revealed an interplay between Toll-like Receptors (TLR) and Interferon (IFN) networks. Enrichment analyses and hierarchical clustering revealed that this relationship is consistently triggered in peripheral white blood cells, peripheral blood mononuclear cells, and dendritic cell transcriptomes, involving IFN- γ , IFN- α/β - (e.g., ISGs, IRFs, SOCS, and GBPs) and TLR-associated molecules (TLR3,4,7/8,9, and TRAF-mediated NF- κ B). These TLR- and IFN-associated genes cluster and increase their correlation levels after Candida stimulation, forming a highly interconnected interferome network, which contains an immune overlap with the anti-viral responses. Notably, our analysis shed new lights on the molecular basis of several genes associated with inborn errors of immunity that cause host susceptibility to fungal infections such as Candida spp., which reinforce our transcriptomic findings.

INTRODUCTION

Fungal infections, including the emergence of new fungal pathogens highly resistant to antifungal drugs, represent a major global health issue¹⁻⁵. Fungal infections affect over a billion people worldwide and kill more than 1.5 million individuals annually. This mortality rate is similar to that of tuberculosis and 3-fold higher than that of malaria^{6,7}. Invasive candidiasis (IC) is the most common fungal disease, affecting approximately 250,000 people annually and causing more than 50,000 deaths^{8,9}. The increasing number of patients with human immunodeficiency virus (HIV) infection, malignancies, inborn errors of immunity (IEI), autoimmune diseases (receiving immunosuppressive treatment), and hematopoietic stem cell or organ transplant recipients contributes to this high frequency of individuals susceptible to life-threatening fungal pathogens^{10,11}. The severity of fungal infections in healthy subjects ranges from asymptomatic or mild mucocutaneous manifestations to life-threatening systemic infections. These data reinforce the critical role of interactions between the microbial and host immune system in the outcome of infection^{7,12}, which needs to be further investigated to find new therapies to reduce morbidity and mortality caused by *Candida* infections^{13,14}.

The critical role of molecules involved in the anti-fungal immune response has been shown by studying patients suffering from IEI, which often result in increased host susceptibility to fungal infections¹⁵. These *in natura* examples, together with *in vitro* and *in vivo* studies have demonstrated the complexity of the anti-fungal immune responses¹⁶⁻¹⁸. For instance, the first steps of anti-fungal immune response involve the appropriate recognition of pathogen-associated molecular patterns (PAMPs) by different pattern recognition receptors (PRRs) expressed on the cell membrane such as C-type lectin receptors (CLRs:

87 dectin 1, dectin 2, and CD209), scavenger receptors (CD36), and toll-like receptors (TLRs:
88 e.g., TLR2 and 4). Intracellular PRRs including RIG-I-like receptors (RLRs: melanoma
89 differentiation-associated protein 5 or MDA5), TLRs (e.g., TLR3 and TLR9), and NOD-like
90 receptors (NLRs: nucleotide-binding oligomerization domain-containing protein or NOD1/2,
91 NOD-, LRR- and pyrin domain-containing 3 or NLRP3) are also relevant and expressed by
92 antigen-presenting cells and phagocytes, which bind to well-known ligands^{16,18,19}. Activation
93 of PRRs induces several signaling events such as the canonical Nuclear factor (NF)- κ B
94 pathway²⁰ that trigger effector anti-fungal mechanisms such as phagocytosis, production of
95 reactive oxygen species (ROS)²¹, degranulation, and neutrophil extracellular traps
96 (NETs)^{22,23}. Simultaneously, PRRs promote the production of key inflammatory cytokines
97 such as tumor necrosis factor (TNF)- α , Interleukin (IL)-1 β , IL-6, IL-17, type I Interferons
98 (IFNs [IFN- α/β]), and the IL-12/IFN- γ axis^{18,24-26}, which shape and instruct immune cells.
99 Detailed mechanism and pathways involved in the immune defense against candida
100 infections have been reviewed elsewhere²⁷.

101 Whole transcriptome profiling technologies have revolutionized the biomedical
102 sciences, opening avenues to identify new signaling pathways, biomarkers, and therapeutic
103 targets^{28,29}. The association between PRR activation and cytokine production by immune
104 cells has been important and has been abundantly investigated by linear approaches or
105 strategies designed to identify the anti-fungal transcriptomic signature^{12,26,30,31}. However,
106 none of these investigations has assessed the interactome of anti-fungal molecules in a
107 holistic, integrative way. To reach this goal, we employed a stepwise, integrative, and systems
108 immunology approach to investigate publicly available transcriptome data of anti-Candida

immune responses. Here, we characterized the functional genomic landscape of the immune responses to *C. albicans* and *C. auris*.

RESULTS

Modular gene co-expression analysis reveals an interplay of TLR- and IFN-associated genes

We first aimed to uncover the functionality and interaction involved in the development of anti-fungal immune responses and their immunomodulatory compounds at the systems level. To reach this goal, we performed modular gene co-expression analysis³², which has not been employed previously to understand the immune response to fungal pathogens. For this analysis, we used the dataset from Smeekens et al. (GSE42606)³³, a unique public dataset available containing more than 15 samples per group (30 uninfected and 24 *C. albicans*-activated samples), which is required to obtain biologically meaningful modular networks³⁴. Modular gene co-expression analysis using CEMiTool³⁵ identified thirteen enriched co-expression modules from the total expressed genes by peripheral blood mononuclear cells (PBMCs; which contain lymphocyte subpopulations, monocytes, and dendritic cells) (**Fig.1a**). Among these modules, 12 were significantly affected (9 downregulated and 3 upregulated) in response to *C. albicans* infection. Of note, modules M1 and M2 indicate gene co-expression and upregulation of Interferon and Interleukin signaling with TLR cascades (**Fig. 1b-e**). These results suggest an interrelated and interdependent relationship (interplay) between the TLR and IFN signaling cascades during the anti-Candida immune response activation.

Functional clustering of TLR- and IFN-associated genes

Based on the results obtained by the modular co-expression analysis, we dissected the significantly enriched pathways from differentially expressed genes (DEGs) induced by *C. albicans*³³. In agreement with the topological results obtained using CEMiTool, over-representation analysis (ORA) of DEGs using the ClusterProfiler tool³⁶ pinpointed different clusters related to the activation of the TLR and IFN signaling cascades (**Fig 2a-b**). IFN signaling was the most enriched pathway modulated by *C. albicans*. The relationship between the 30 most enriched pathways and their associated genes is shown in a network view (**Fig 2c**) while the entire list of all enriched pathways is summarized in **Suppl. Table 1**. *C. albicans* activation significantly enriched chemokine (G protein-coupled receptors [GPCR] ligand binding) and cytokine signaling pathways (IL-10, IL-3 and IL4), IFN- α/β signaling, Interferon-stimulated gene 15 (ISG15) antiviral mechanism, TNF Receptor Associated Factor 3 (TRAF3)-dependent IRF activation, DExD/H-Box Helicase 58 (DDX58)/Interferon Induced with Helicase C Domain 1 (IFIH1)-mediated induction of IFN- α/β , and regulation of type I and II IFN among the IFN signaling events (**Fig 2a and c; Suppl. Table 1**). ORA also indicated that *C. albicans* activates several TLR signaling events such as TLR4, TLR3, TLR7/8, and TLR9 as well as MyD88/TIR-domain-containing adapter-inducing interferon- β (TRIF)/TIR Domain Containing Adaptor Protein (TIRAP) cascades, and TRAF6-mediated NF- κ B activation. **Suppl. Table 2** lists TLR- and IFN-associated genes detected by CEMiTool and ORA.

C. albicans infection activates common TLR- and IFN-associated genes in peripheral blood leukocytes

These results suggest that the activation of PBMCs with *C. albicans* is marked by a previously unreported comprehensive interplay between TLR and IFN signaling pathways. Thus, we next investigated which DEGs and signaling pathways are consistently activated by *C. albicans* in peripheral blood leukocytes such as PBMCs (datasets GSE42606 and GSE154911) and peripheral white blood cells (WBCs, datasets GSE65088 and GSE114180) throughout all publicly available datasets. WBCs contain PBMCs (lymphocytes 20-45% and monocytes 2-10%) and granulocytes (neutrophils: 50-70%; basophils: 0-1%; and eosinophils: 1-5%)³⁷. A meta-analysis of WBC and PBMC gene expression datasets using P-value combination method revealed among the meta-significant genes 44 commonly activated DEGs (40 upregulated and 4 downregulated) (**Fig 3a, Suppl. Table 3**). According to the cell population, these DEGs form well-defined hierarchical clusters, i.e., PBMC datasets present a closer expression pattern among them as well as the WBC datasets, when we compare both regulation and significance (**Fig 3b**). Enrichment analyses using EnrichR of these 44 genes revealed 87 significantly affected pathways (**Suppl. Table 5**), including TLR and IFN- α/β signaling pathways (**Fig. 3c**). Furthermore, these 44 DEGs also enrich other related interleukin signaling pathways such as JAK-STAT, IL-12, IL-17, IL-23, TNF, and chemokines (GPCR ligand binding) and PRRs, including RIG-I like receptor and NOD signaling. Multi-study Factor Analysis of eligible³⁸ datasets (WBC: GSE65088 and PBMCs: GSE42606; those with minimal number of samples required for this analyses) identified two common latent factors with high loadings, while specific latent factors showed low loadings across these studies. Thus reinforcing the biological relevance of these 44 common genes (**Fig. 3d, Suppl. Table 7**).

***C. albicans* activate common TLR and IFN signaling pathways across different layers of immunity**

The analysis presented above supports the idea that WBCs and PBMCs consistently co-modulate TLR and IFN signaling cascades in response to *C. albicans*. Subsequently, we added monocyte-derived dendritic cells (moDCs) datasets (GSE77969, E-MTAB-135, E-MTAB-751) into our integrative analysis. moDCs are known to be essential players of anti-fungal immunity, bridging the immune system's innate and adaptive arms. We searched for genes commonly regulated by *C. albicans* in transcriptomes of WBCs, PBMCs, and moDCs, in uninfected or *C. albicans* activation conditions. Intersection analyses performed according to cell population identified 123, 223, and 57 common DEGs among WBC, PBMCs, and moDCs datasets, respectively (**Fig. 4a-c**). However, when we compared the seven datasets together, we found only 2 common DEGs (**Fig. 4d**), which by themselves do not significantly enrich signaling pathways. We then asked if DEGs from each dataset enrich common signaling biological processes among all studies. Gene Ontology (GO) analysis using ClusterProfiler analysis revealed 173 common biological processes (**Suppl. Table 8**). We found several molecules/pathways essential for the anti-fungal immune response³⁹. Among them, there is a cluster of IFN- γ , and NF- κ B signaling, and a previously described overlap with the immune response to virus (IFN- α/β)²⁶ (**Fig. 4e**). Additional ORA of DEGs involved in this cluster found significant enrichment of several interleukin signaling pathways such as IL-1, IL-4/IL-13, IL-6, IL-10, IL-17, and IFN- α/β as well as signaling cascades of single TLRs (TLR2, TLR3, TLR4, TLR5, TLR9, TLR9, and TLR10), TLR heterodimers (TLR1/TLR2, TLR2/TLR6, TLR7/8), and TLR adapter molecules (MyD88/TIRAP, TRAF6, TRIF) (**Fig. 4f**). **Fig 4g** shows the interactome of some of these 1096 DEGs (**Suppl. Table 12**) enriching common signaling pathways among WBC, PBMC, and moDC datasets. We found 1096 DEGs (**Suppl. Table 12**) affecting the

common biological processes among WBC, PBMC and moDC previously described. **Fig. 4g** shows the interactome obtained from some of these 1096 DEGs and enriched signaling cascades (**Suppl. Table 13**), thus highlighting the association of TLR- and IFN-signaling cascades, which were consistently enriched during our analyses. These 1096 DEGs also enrich other PRR and Interleukin signaling pathways such as CLRs (dectin 1), NLRs (NOD1/2), pro- (IL-1, IL6, IL-17, IL-12), anti-inflammatory (IL-10), and T helper 2 (IL-4 and IL-13) cytokines. This immunological balance between pro-and anti-inflammatory event is crucial for the proper control of fungal infections while maintaining immune homeostasis^{40,41}.

***C. albicans* infection increases the correlation between TLR- and IFN-associated genes**

After verifying TLR and Type I and II IFN signaling cascades' consistency, we assessed the degree of association between these two variables during the immune response to *C. albicans*. Due to minimum sample size requirement⁴², we selected TLR and IFN-associated genes present in the PBMC transcriptome data from Smeekens et al. (GSE42606)³³. This dataset contains 45 and 14 TLR- and IFN-associated DEGs modulated by *C. albicans* when compared to the uninfected group. *C. albicans* infection increased mainly positive correlations between TLR- and IFN-associated DEGs when compared to the uninfected group (**Fig. 5a-5d**). We performed canonical-correlation analysis (CCA) to further assess the association's strength between TLR and IFN DEGs. CCA is a generic parametric model used to quantify relationships between two groups of interrelated and interdependent variables⁴³. This approach unveiled a pair of canonical variates (x-CV1 and y-CV1) highlighting the strong association between most of IFN-associated DEGs with TLR-associated DEGs independently of cell culture (uninfected versus *C. albicans* infected PBMCs) condition (**Fig. 5e, Supp. Fig.**

1). Additionally, this approach revealed a clear stratification of uninfected and *C. albicans*-infected group, based on the pair of canonical variates (**Fig. 5f**). Thus, reinforcing the relevant relationship between TLR- and IFN-associated DEGs in the anti-Candida immune response.

The multidrug-resistant *C. auris* also induces the interplay between TLR and IFN signaling pathways

Considering the emergence and global threat of multidrug-resistant *C. auris*^{31,44}, we also investigated the interplay between TLR and IFN signaling pathways in response to this Candida species. We used the unique publicly available dataset analyzing the immune response to *C. auris* and *C. albicans* (GSE154911). Similar to *C. albicans* activation, ORA of DEGs induced by *C. auris* included TLR and IFN signaling cascades among the 30 most enriched pathways (**Fig. 6a and 6b; Suppl. Table 16 and 17**). *C. albicans* and *C. auris* similarly modulated DEGs' levels involved in TLR signaling, including NF- κ B1, NF- κ B2, JUN, and DUSP4, as well as IFN signaling such as IRFs, GBPs, SOCS1, ISG20, TRIM, and IFIT3 (**Fig. 6c**). When we compared the DEGs induced by *C. auris* with those enriching common pathways among all datasets (1096 DEGs, **Suppl. Table 12**) assessing the immune response to *C. albicans*, we identified 237 common DEGs (**Fig. 6d**). ORA of these common DEGs revealed that the interplay between TLR and IFN signaling cascades is a consistent immunologic feature in response to these two Candida species (**Fig. 6e, Suppl. Table 18**).

Inborn errors of immunity (IEI) corroborate the interplay between TLR and IFN signaling cascades

Finally, we aimed to evaluate the potential clinical and translational relevance of the TLR- and IFN-associated genes and molecular pathways consistently modulated by *Candida*. Therefore, we searched for IEI associated genes that are known to increase human susceptibility to fungal infections. So far, mutations in 100 genes have been associated with IEI that cause increased susceptibility to candidiasis and often other clinical manifestations. We compared them with the 1096 genes (**Suppl. Table 12**), i.e., those enriching the common biological processes activated by *Candida* (**Fig. 4**). These 1096 genes encode molecules present in different cellular compartments such as extracellular regions, organelles, and nuclei and those forming macromolecular complexes. Together, they form a highly interconnected physical protein-protein interaction network (**Fig. 7a**), which contains several hubs⁴⁵ (**Fig. 7b**), here defined as those having more than or equal to 200 interaction partners. Of note, 34 genes associated with IEI are also present across the studies. Meanwhile, although 66 genes associated with IEI are not identified in the datasets, these genes are highly connected with the other DEGs in this network. Furthermore, the 1096 DEGs mostly contain Type I and II IFN- associated genes (**Fig. 7c, Suppl. Table 19**).

Of note, we identified in this network a total of 34 genes associated with 7 groups of IEI, including congenital defects of phagocytes, defects of intrinsic and innate immunity, predominantly antibody deficiencies, and diseases of immune dysregulation, as defined by the International Union of Immunological Societies Expert Committee(IUIS)⁴⁶ (**Fig. 8a**). Notably, among the hubs are STAT1, STAT3, NFKBIA ($\text{I}\kappa\text{B}\alpha$), and NFKB1, which are well known to be associated with TLR and IFN signaling pathways⁴⁷⁻⁵². ORA of these 34 genes indicates that in addition to dectin-1⁵³ and NLRs signaling. They mostly enrich both type I and II IFN and several TLR signaling pathways (TLR1/2, TLR2/6, MyD88, and TRAF6-

mediated NF- κ B activation) (**Fig. 8b-c, Suppl. Table 20**). Taken together, these data strongly support the immunobiological relevance of the interplay between TLR and IFN signaling cascades (**Fig. 8d**).

DISCUSSION

So far, several immunologic molecules and pathways such as those triggered by TLR and IFN, which induce the generation of T cell subpopulations (e.g., T helper 1 [Th1], Th17, and T regulatory [T reg] cells) have been successfully characterized by individual studies and mechanistic approaches¹⁸. However, until now, there was no system immunology study to holistically understand the anti-fungal immune responses. Our approach integrates dispersed transcriptomic datasets that investigated the immune response against *C. albicans* and *C. auris*, indicating that the anti-candida immune responses are marked by a previously uncharacterized intricate interferome chain, interconnecting PRR (e.g., CLRs, TLRs, and NLRs) and interleukin (e.g., IFN, TNF, and IL-10) cascades. This immune network is hallmarked by dynamic and consistent crosstalks between the network interplay of TLR and IFN signaling pathways. Besides, we show that there is a consistent overlap between the antiviral and antifungal immune responses, which supports the previously reported pivotal role of IFN type I in the immune response against *C. albicans*²⁶. Notably, this immunologic overlap might be not restricted to viral infection. For instance, studies need to be performed to investigate its extension to the anti-*Mycobacteria tuberculosis* immune responses. The host's protection against this intracellular bacterium relies not only on an IFN- γ centered phenomenon but also requires the synergism of Type 1 IFNs and other cytokines such as IL-17⁵⁴. Thus, our results strongly suggest that the immune systems employ a multitude of

molecules, working as a “social” network, in which cells effectively collaborate and communicate to maintain immunologic homeostasis^{55,56}.

Our integrative and systems immunology approach provides a transcriptomic landscape of the anti-candida defenses that will contribute to better understand the host immunological dynamics initiated against these fungal pathogens. Since we currently are confronted with increasing numbers of invasive fungal infections, in part due to the emergence of anti-fungal drug resistance, it is imperative to address this global health problem^{2,44,57}. Diseases caused by viruses and bacteria have been recognized as important public health issues for centuries, while fungal infections have historically been neglected⁵⁸. Most investigations addressing the anti-fungal immune response employ linear and mechanistic approaches¹⁶ such as numerous transcriptomic, epigenomic, and proteomic data available that investigate the immune response against viruses and bacteria using public databases^{59–61}. There are very limited numbers of publicly available transcriptomic datasets of human immune responses to fungal infections. Most of these studies focus on the transcriptomic response of human immune cells activated with *C. albicans* but only one dataset explores the immune response to *C. auris*, one of the most critical emerging fungal pathogen³¹. The integrative approach we employed was designed to obtain a comprehensive understanding of the anti-fungal immune response.

The results of our study provide complementary arguments for linear and mechanistic strategies that confirm a dynamic interplay between TLR and type I and II IFN-associated molecules⁶². It has been suggested that different TLRs synergistically activate immune cells to, for instance, induce the expression of several proinflammatory molecules through the cooperation of NF- κ B, IRF, STAT, MAPK, ITAM, and PI3K signaling pathways^{63–65}. On the one

hand, TLR-induced NF- κ B signaling promotes the production of several key cytokines including IFNs that activate STAT1-mediated signaling pathway⁶⁶. On the other hand, IFN- γ increases the expression of genes encoding TLRs⁶⁷⁻⁷⁰. IFNs also potentialize TLR-induced gene transcription by creating a primed chromatin environment by histone acetylation that allows sustained occupancy of transcription factors STAT1 and IRF-1 at promoters and enhancers at the *TNF*, *IL-6*, and *IL12B* loci⁷¹. Thus, our phenomenological study confirms these previously reported mechanistic studies and provides new insights into the molecular network of TLR and IFN signaling pathways, which also need to be investigated in other mycoses (paracoccidioidomycosis, histoplasmosis, and cryptococcosis) and other neglected diseases (Dengue, Zika, leishmaniasis, and Chagas disease) occurring in developing countries⁵⁸. The TLR and IFN interactome involve more complex events than previously thought, demanding further bottom-up and top-down systems immunology investigations.

Our conclusions are based on the integration of publicly available human transcriptome that identified common DEGs, as well as biological processes and signaling pathways consistently modulated in WBCs, PBMCs, and moDCs in response to fungal pathogens (*C. albicans* and *C. auris*). Among these DEGs, we highlight those involved in IFN- α/β (e.g., ISGs, IRFs, SOCS, and GBPs), TLR3,4,7/8,9, and TRAF-mediated NF- κ B signaling cascades. The correlation levels of DEGs involved in these signature clusters increased upon stimulation with *C. albicans*. Of note, among the consistently identified DEGs are those previously associated with IEI that increase the host susceptibility to fungal infections such as those causing chronic mucocutaneous candidiasis, which supports our study's findings and increases the understanding of consistent immunologic pathways essential for the immune response to Candida infections.

However, confirming and validating the translational relevance of these complex signaling events is challenging. In this regard, our consistent finding, using several transcriptomic datasets, of a strong interconnection between TLR-IFN signaling, indicate the fundamental role of this systemic interplay against *C. albicans* and *C. auris*, the most important²⁷ and multidrug-resistant fungal pathogens^{44,72,73}. Interestingly, several of the consistently modulated DEGs and pathways in response to *C. albicans* and *C. auris* have been associated with several IEI¹⁵. Besides, these DEGs are also involved with immunological pathways related to the development of IEI phenocopies such as those targeted by anti-IL-17 or anti-IL17RA autoantibodies that result in increased susceptibility to *Candida* spp. infections. This observation supports the importance of our integrative and systems immunology approach. Because the outcome of fungal infections depends primarily on the host immune response, it is most relevant to review those IEIs that predispose to *Candida* infections^{27,74}. IEIs represent an essential research field that investigates natural human susceptibility models to infection, often revealing the non-redundant role of genes involved in immunologic homeostasis⁷⁵⁻⁷⁷. Of the 416 molecular defined IEI recently summarized by the expert committee of the IUIS, more than 20 syndromes were recognized to be associated with susceptibility to fungal infections⁴⁶. This list of genes associated with increased risk of fungal infections includes genes regulating signaling via the IL-2 receptor, via NF- κ B activation, IFN induced signaling, activation of STATs, and TLR signaling. Thus, these observations support the relevance of the interactome and interplay events characterized by our analysis.

All in all, our work provides a systems immunology view of the interactome of anti-fungal molecules, revealing a consistent network interplay between TLR and IFN signaling

pathways in response to *C. albicans* and *C. auris*. This study also indicates new biomarkers and provides novel insights into the systemic immunological mechanism against fungal infections. Future works dissecting this interplay will pave the way for new immunotherapy approaches to reduce the high mortality rate caused by fungal infections. Also, our study indicates that the exploration of functional genomic approaches by applying systems immunology methods to investigate IEI will provide new opportunities to further understand the immune system *in natura*.

ONLINE METHODS

METHODS

Datasets and curation

We performed an integrative analysis by searching on NCBI GEO database⁷⁸ and ArrayExpress database⁷⁹, to identify publicly available gene expression data of infection by *C. albicans* and *C. auris* in whole blood, PBMCs, and moDCs. This search comprised studies published between March 2010 and July 2020. Since transcriptome datasets from patients with candidiasis were not publicly available we consider as criteria for inclusion: (1) gene expression data of whole blood, PBMCs, and moDCs of in vitro infection with *C. albicans*; (2) studies composed of at least 2 samples per group; (3) the inclusion of control groups for comparison; (4) all gene expression analysis platforms were considered; and (5) only studies that have provided the transcriptome data were included for the integrative analyses. Our exclusion criteria were (1) non-human samples, (2) treatment before molecular genetic analysis, and (3) review studies. RNAseq and MicroArray studies were included in our integrative analysis, five studies were retrieved from the NCBI GEO database⁷⁸ (GSE65088

and GSE114180, GSE42606, GSE154911, and GSE77969) and two from ArrayExpress database⁷⁹ (E-MTAB-135, E-MTAB-751).

Differential Expression Analysis

To characterize the immunological signature from the global transcriptional profiles in infection by *C. albicans*, read counts of each RNAseq study were transformed (log2 count per million), and NetworkAnalyst 3.0 webtool (<https://www.networkanalyst.ca/>)⁸⁰ was used to perform differential expression analysis, applying DESeq2 pipeline. The microarray studies were analyzed through GEO2R web application⁸¹, available at <http://www.ncbi.nlm.nih.gov/geo/geo2r/>, using limma-voom pipeline⁸². To select the up and downregulated genes between *C. albicans* infection and the normal group, we used the statistical cutoffs of log2 fold-change > 1 (upregulated) or < -1 (downregulated) and adjusted p-value < 0.05.

Analysis of Gene Co-Expression Modules

We selected the dataset GSE42606 to analyze the gene co-expression modules with the R-package CEMiTool 1.12.2 using default parameters³⁵.

Enrichment Analysis and Data Visualization

We used the differentially expressed genes (DEGs) to identify enriched ontology terms. The pathways and the biological processes were identified through an Over-representation Analysis (ORA) and EnrichR⁸³, and the significant enriched immunological terms were

generated according to adjusted p-value < 0.05. The Upset and Venn Graphs demonstrating the intersections and comparisons between common DEGs among the datasets were generated through the webtool Intervene⁸⁴ and Bioinformatics & Evolutionary Genomics (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). We plotted the set of genes shared between the dataset in bubble-based heat maps, applying One minus cosine similarity through the webtool Morpheus (<https://software.broadinstitute.org/morpheus/>)⁸⁵. We used ClusterProfiler⁸⁶ to obtain dot plots of enriched terms associated with *C. albicans* and *C. auris* infections. ClusterProfiler and ORA, were performed on R software version 4.0.2 (<https://www.r-project.org/index.html>), through the packages DOSE, enrichplot, reactomePA, and clusterprofiler⁸⁶. The GOpot was plotted using the R packages unkn, circlize, and GOpot⁸⁷. The statistical graphs were constructed using the functionalities of the ggplot2 package⁸⁸. We represented the shared DEGs between different fungal infections (*C. albicans* and *C. auris*) through circular heatmaps, using the R packages circlize and ComplexHeatmap⁸⁹.

Correlation Analysis

We used the GSE42606 dataset to perform the correlation analysis between genes associated with TLRs as well as Type I and II IFN signaling cascades. The correlation matrices were generated with the webtool Intervene⁸⁴ (<https://intervene.readthedocs.io/en/latest/index.html>), using Pearson coefficient. The Canonical Correlation Analysis (CCA)⁹⁰ was applied to investigate patterns of association between IFN and TLR genes from the same dataset. The CCA was performed on R software

version 4.0.2 (<https://www.r-project.org/index.html>), through the packages DFA, CANCOR, and whitening⁹⁰.

Molecular Network

Networks of related pathways to fungal infection immune responses and physical protein-protein interaction (PPI) networks of DEGs found across all datasets were annotated, analyzed and visualized using NAViGaTOR 3.0,¹⁴⁹¹. Node color represents Gene Ontology cellular component as per legend. DEGs were used as input into Integrated Interactions Database (IID version 2020-05; <http://ophid.utoronto.ca/iid>)^{92,93} to identify direct physical protein interactions. Networks were exported in SVG file format, and finalized in Adobe Illustrator 2021.

Multi-study Factor Analysis (MSFA)

MSFA is a generalized version of factor analysis that allows for the joint analysis of multiple studies. MSFA estimates shared factors common to all studies, as well as factors specific to individual studies. Estimation of parameters for the MSFA model can be computed using either a frequentist or a Bayesian approach. Compared with the frequentist analysis, the Bayesian offers two major advantages: 1- it provides a better defined factors, and 2- it chooses the dimension of the common and study-specific factors through a practical and useful approach. We adopt the Bayesian multi-study, for the inferential analysis to identify common and study-specific factors^{27,38,94} shared by GSE65088 and GSE42606. The Bayesian

MSFA considers all data at once in an integrated approach, estimating parameters by maximum-likelihood analysis⁹⁵.

Interferome Analysis

The identification of interferome genes was performed with Interferome V2.01 (<http://www.interferome.org/interferome/home.jspx>).

Code Availability

R codes used in this work are available at https://github.com/ranieri131/SalgadoRC_CANDIDA_IMMUNE_RESPONSE_2021

Acknowledgments

We acknowledge the São Paulo Research Foundation (FAPESP grants 2018/18886-9, 2020/01688-0, and 2020/07069-0 to OCM) for financial support. RCS is financed by the national council for scientific and technological development (CNPQ). Computational analysis was supported by FAPESP and partially by the grants from Ontario Research Fund (#34876), Natural Sciences Research Council (NSERC #203475), Canada Foundation for Innovation (CFI #29272, #225404, #33536), and IBM. This study was financed in part by the coordination for the improvement of higher education personnel – Brazil (CAPES) – finance code 001.

Author Contributions

RCS, PPF, OCM co-wrote the manuscript; RCS, DLMF, TTF, PPF, NOSC, VLGC, LFS, OCM provided scientific insights; RCS, DLMF, AHCM, SMSN, KTA, CASP, GCB, DRP, ISF, RV, IJ, and OCM performed bioinformatics analyses; RCS, DLMF, and OCM conceived and designed the

study; RCS, TTF, LFS, PPF, NOSC, VLGC, HDO, LFS, IJ, ACN, and OCM revised and edited the final manuscript; ACN and OCM supervised the project.

Competing interest statement

The authors declare no competing financial and/or non-financial interests concerning the work described.

Data availability

The published transcriptome datasets can be found in the GEO and Array Express databases (IDs. GSE65088, GSE114180, GSE42606, GSE154911, GSE77969, E-MTAB-135, E-MTAB-751).

Figure Legends

Fig. 1. Modular gene co-expression analysis and the association of TLR and IFN signaling pathways. **a**, Co-expression modules significantly enriched (M1-M11, and M13) in PBMCs (uninfected n= 30; *C. albicans* infected n= 24; dataset GSE42606). **b** and **d** Network representation of M1 and M2 with hubs (most connected genes) colored based on co-expression (blue color), co-expressed and interactions (green color), or only interactions (dark-red color). **c** and **e**, Enrichment representation obtained by modular genes co-expression in M1 and M2 showing significantly ($-\text{Log}_{10}$ transformed adjusted p-value) enriched signaling pathways.

Fig. 2. Functional clustering of differentially expressed genes (DEGs) associates TLR and IFN signaling pathways. **a**, Dot plot showing the 30 most enriched signaling pathways obtained by over-representation analysis (ORA) of DEGs (dataset GSE42606) using ClusterProfiler. Y-axis contains enriched pathways while the size of circles represents the number of genes (count) enriching each category and color (from blue to black) indicates how significant (when p-value < 0.05) enriched is the pathway. **b**, Network of TLR- and IFN-associated genes enriching signaling pathways showed in the dot plot (a). The network includes genes upregulated and downregulated (**Suppl. Tabl. 1**) when comparing uninfected with *C. albicans*-infected PBMCs. The size of circles represents the number (counts) of genes that enrich the pathway, and colored squares represent the cell location of genes. **c**, Network demonstrating interactions between pathways and their associated genes revealed by ORA. Circular nodes represent pathways and their size is associated with the number of genes that enrich them, while colored squares represent the cell location of genes. The interaction network was build using the NAViGaTOR software.

Fig. 3. *C. albicans* activates common TLR- and IFN-associated genes in peripheral blood leukocytes. WBC and PBMCs. **a**, The upset plot displays the number (set size) of DEGs present in each dataset (y-axis: WBCs, GSE65088, and GSE114180; PBMCs: GSE42606 and GSE154911) and their intersections. Black bubbles, present in the rows, mark the data set which refers to the amount present in the blue columns, with intersections between more than one group being shown. **b**, Bubble heatmap, generated with Morpheus, demonstrates gene expression patterns across the different studies. The size and color of circles correspond to $-\text{Log}_{10}$ transformed adjusted p-value and Log_2 fold change (Log_2FC), respectively (**Suppl. Table 4**). Blue represents downregulated and red indicates up-regulated DEGs. The cut-off applied to identify the upregulated genes was $\text{Log}_2\text{FC} > 1$ and adjusted p-value < 0.05. Rows and columns were clustered based on cosine similarity between Log_2FC values. **c**, GOplot of selected immunological pathways and genes associated. All pathways are present in **Suppl. Table 6**. **d**, Heatmaps of common and specific latent factors across the studies. Heatmaps contain genes presenting positive and negative loadings ranging from -1 to 1.

Fig. 4. *C. albicans* activate common TLR and IFN signaling pathways across different layers of immunity. **a-c**, Venn diagrams displaying the number of DEGs present in each group of cells and their intersections: white blood cells or WBCs (a), peripheral blood mononuclear cells or PBMCs (b), and monocyte-derived dendritic cells or moDCs (c). **d**, The plot highlights the number of common DEGs across individual cell groups. **e**, Bubble heatmap exhibiting the genes enriching common biological processes across the studies (**Suppl. Table 9 and 10**). **f**, Further analysis of TLR- and IFN- associated

pathways. In both heatmaps the size of circles corresponds to adjusted p-value transformed into -Log10 and color intensity indicates the number of genes in each biological process and pathway across the studies, respectively (**Suppl. Table 11**). **g**, Network demonstrating the interactions between TLR- and IFN-associated DEGs/signaling pathways with other molecules and signaling cascades classically associated with the antifungal immune responses. Enrichment analysis was performed using Reactome. Circular nodes represent pathways and their size denote the number of genes enriching the pathways. Colored squares represent the cellular location of genes. The interaction network was build using the NAViGaTOR software.

Fig. 5. Relationship between molecules associated with TLR and IFN signaling cascades. a,b, Correlplot of genes associated with TLRs and IFN signaling cascades in PBMCs (GSE42606) in the **a**, absence or **b**, presence of *C. albicans*. Histograms of Pearson's correlation coefficient, containing negative and positive correlation from 1 to -1, respectively. **c,d**, The spectrum of correlations in the **c**, absence or **d**, presence of *C. albicans* (**Suppl. Table 14 and 15**). **e**, Estimated correlations of IFN- and TLR- associated genes versus their corresponding first 2 canonical variates (x-CV1 and x-CV2, for IFN-associated genes; y-CV1 and y-CV2 for TLR-associated genes). Grey colored variables (with names omitted) are those with canonical correlation coefficient <0.7 in its two corresponding canonical variates. Inner dotted lines limit the canonical correlation coefficient between -0.7 and 0.7, while outer dotted lines between -1 and 1. **f**, Stratification of uninfected and *C. albicans* infected PBMCs based on canonical scores, according to IFN- and TLR-associated genes.

Fig. 6. Induction of the interplay between TLR and IFN signaling pathways by the multidrug-resistant *C. auris*. **a**, Dot plot showing the 30 most enriched signaling pathways obtained by overrepresentation analysis (ORA) of DEGs using ClusterProfiler. Y-axis contains enriched pathways while the size of circles represents the number of genes (count) enriching each category and color (from blue to black) indicating how significantly enriched (when p-value <0.05) is the pathway. **b**, Network demonstrating interactions between pathways and their associated genes revealed by ORA. Circular nodes represent pathways and their size is associated with the number of genes that enrich them, while colored squares represent the cell location of genes. The interaction network was build using the NAViGaTOR software. **c**, Circular heatmaps of RNAseq expression z-scores computed for log 2 transformed DEGs (p-value adj < 0.05, foldchange > 1 and < -1) compares the expression of TLR- (left panels) and IFN- (right panels) signaling pathways induced by *C. albicans* (green/grey heatmaps) or *C. auris* (yellow/grey heatmaps) all from GSE154911. Small circular heatmaps (blue/grey) demonstrate common DEGs moduled by *C. abicans* and *C. auris*. This is a comparison between *C. albicans*- and *C. auris*-modulated genes (GSE154911). **d**, Venn diagram showing the transcriptional overlapping (an intersection containing 237 shared DEGs) induced by *C. auris* and *C. albicans* (those 1096 genes found across all studies: **Supp. Table 19**). **e**, Dotplot of enriched pathways by the 237 shared DEGs.

Fig. 7. The interactome of DEGs enriching signaling pathways involved in the anti-candida immune response and its association with inborn errors of immunity. **a**, Relationships (edges) among the 1096 DEGs (nodes) found across all studies (**Supp. Table 12**). Subnetworks (semicircles) represent genes associated with IEI causing increased susceptibility to candidiasis, being 34 purple nodes genes shared with the group of 1096 DEGs, while 66 green nodes represent those not found in

the *Candida* datasets. Colored squares and circles represent the cell location of genes. The interaction network was built using the NAViGaTOR software. **b**, Network of hubs present in **a**. **c**, Venn diagram of interferon types associated with the group of 1096 DEGs. Interferome analysis revealed IFN-regulated genes modulated either by IFN type I, II, and III, as in the Venn Diagram.

Fig. 8. Inborn errors of immunity confirm the interplay of TLR- and IFN-associated genes. **a**, Overview (created using BioRender.com) of genes associated with inborn errors of immunity that are also found among the DEGs across the 7 datasets of WBCs, PBMCs, and moDCs (**Suppl. Table 21**). **b**, Dot plot showing the 30 most enriched signaling pathways obtained by over-representation analysis (ORA) of DEGs seen in (**a**). Y-axis contains enriched pathways while the size of circles represents the number of genes (count) enriching each category and color (from blue to black) indicates how significantly enriched (when p-value <0.05) is each pathway. **c**, GOPlot displaying genes causing inborn errors of immunity and enriched pathways. **d**) Schematic view (created using BioRender.com) datasets used in this study (left panel) and a summary of main interplays between TLR and IFN signaling pathways in the immune response to *C. albicans* (right)^{52,96–102}.

Supplementary Figure 1. Heatmap of Pearson correlations between TLR- and IFN- associated genes. Gene versus gene correlation was assessed placing gene names as column while culture conditions as rows. Thus, uninfected and *C. albicans* activated were analyzed as a unique group (**Suppl. Table 22 and 23**).

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8