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**Candida albicans** Dampens Host Defense by Downregulating IL-17 Production

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IL-17 is one of the key cytokines that stimulate host defense during a *Candida* infection. Several studies have demonstrated the capacity of *Candida albicans* to induce a Th17 response. Surprisingly, experiments employing live *C. albicans* demonstrated a specific downregulation of host IL-17 secretion in human blood mononuclear cells (PBMCs). By avoiding the direct contact of live *C. albicans* and PBMCs, we demonstrate that this inhibition effect is mediated by a soluble factor released by live *C. albicans*. However, this effect is due neither to the releasing of *C. albicans* pathogen-associated molecular patterns nor to the alteration of different Th cell subtypes. Rather, we found that live *C. albicans* shifts tryptophan metabolism by inhibiting IDO expression away from kynurenines and toward 5-hydroxytryptophan metabolites. In addition, we show that these latter 5-hydroxytryptophan metabolites inhibit IL-17 production. In conclusion, live *C. albicans* inhibits host Th17 responses by modulatory effects on tryptophan metabolism. *The Journal of Immunology*, 2010, 185: 2450–2457.

As a commensal pathogen, *Candida albicans* colonizes the mucosal surfaces in healthy individuals without causing/inducing symptoms, yet it can also cause a wide range of different infections ranging from vaginal candidiasis, onychomycosis, oropharyngeal candidiasis, and disseminated candidiasis in situations in which the host defense is decreased, such as in neutropenic patients, patients in intensive care units, or patients with genetic defects. In these patients, mortality due to disseminated candidiasis reaches 40% (1). Host immunity against *C. albicans* is crucial in controlling *C. albicans* infection. The innate immunity is believed to be the first line of host defense, such as the direct killing of yeasts through phagocytosis by neutrophils and macrophages. In addition to innate immune cells, an adjunctive protective effect is played by cellular adaptive immunity represented by Th lymphocytes. The balance of various Th cell subpopulations plays a crucial role in regulating the prognosis of *C. albicans* infection (2).

Apart from conventional Th1/Th2 responses, Th17 cells have recently been described as an important Th cell subtype conferring protection against extracellular bacterial and fungal infections (3). IL-17A, the major cytokine secreted by Th17 cells, possesses multiple proinflammatory functions, such as recruiting neutrophils (4, 5), activating neutrophil/macrophage phagocytosis activity, and inducing β-defensin release (6). Therefore, IL-17 is regarded as an important component in host defense against *C. albicans* infection. Acosta-Rodríguez et al. (7) found that among memory CD4+ T cells from healthy volunteers, *C. albicans*-specific cells are predominantly found in the Th17 subset. Moreover, by comparing healthy volunteers and patients with chronic mucocutaneous candidiasis, it was determined that IL-17 production by peripheral leukocytes was strongly reduced in patients with chronic mucocutaneous candidiasis (8), implying an important role of IL-17 in mucosal host defense against *C. albicans* infection. Similar results were also found in patients with hyper-IgE syndrome (9), which further argues for a critical role of IL-17 in host defense against *C. albicans*. Moreover, it has been proposed that IL-17/IL-17AR is required for normal fungal host defense in systemic *Candida* infection in mice (10). All of these data demonstrate that IL-17 is crucial for host defense against *C. albicans* infection. Recently, the pathway through which *C. albicans* induces IL-17 production has been also identified as the C-type lectin mannose receptor and dectin-2 (11, 12), which is amplified by the TLR2/dectin-1 pathway (12, 13).

As Th17-mediated antifungal pathways are very effective in eliminating the fungus, yet *C. albicans* colonizes the mucosal surfaces of up to 30% of healthy individuals at any given time, we hypothesized that *C. albicans* is also able to modulate host IL-17 production, permitting it to colonize the host. In this study, we demonstrate that this is indeed the case, with live *C. albicans* exerting inhibitory effects on IL-17 production through the modulation of tryptophan metabolism.

**Materials and Methods**

**Reagents**

Pepstatin A, l-tryptophan, 5-hydroxy-l-tryptophan, and l-kynurenine were purchased from Sigma-Aldrich (St. Louis, MO). Mouse anti-human monoclonal anti-TLR2 Ab was purchased from eBioscience (San Diego, CA). Laminarin, a specific inhibitor of dectin-1, was kindly provided by Dr. David Williams (University of Tennessee, Knoxville, TN). Chitin was kindly provided by Prof. Neil Gow (University of Aberdeen, Aberdeen, U.K.) and prepared according to protocols described elsewhere (14). Bar- tonella LPS (anti-TLR4) was obtained as previously described (15).
Volunteers

Blood samples were collected from six healthy nonsmoking volunteers. After written informed consent was obtained, venipuncture was performed to collect blood into 10-mI EDTA tubes (Monoject, Covidien, Mansfield, MA).

C. albicans strain

C. albicans ATCC MYA-3573 (UC 820) (16) was used, unless otherwise indicated. C. albicans organisms were grown overnight in Sabouraud broth at 37°C, and cells were thereafter harvested by centrifugation, washed twice, and resuspended in culture medium (RPMI 1640; ICN Biomedicals, Irvine, CA) (17). C. albicans was killed for 1 h at 100°C and resuspended in culture medium to the final concentration of 10⁶ C. albicans yeasts/ml. C. albicans was inoculated in RPMI 1640 and grown in a 37°C incubator for 24 h.

Isolation and stimulation of PBMCs

Separation and stimulation of PBMCs was performed as described elsewhere (18). Cells were adjusted to a concentration of 5 × 10⁶ cells/ml and thereafter incubated at 37°C in round-bottom 96-well plates (volume, 100 μl/well) with either heat-killed C. albicans (10⁶ microorganisms/ml), live C. albicans, or culture medium. To test the tryptophan metabolites effect, 100 μg/ml L-tryptophan, 5-hydroxy-L-tryptophan, and L-kynurenine were added simultaneously with heat-killed C. albicans. After 7 d, supernatants were collected and stored at −20°C until assayed.

Transwell stimulation experiments

The transwell system is applied as described previously to study the function of soluble factors released by live C. albicans in modulating host immune responses (19). Live C. albicans (10⁶ microorganisms/ml) were cultured in the upper well of the 24-well transwell system (pore size 0.4 μM; Corning, New York, NY) to avoid direct contact between live C. albicans and PBMCs, yet allowing the free diffusion of the released soluble factors. PBMCs were cultured in the lower well and were stimulated with several stimuli as described below (β-glucan, chitin, Bartonella LPS, anti-TLR2 Ab, and pepstatin A).

Cytochrome measurements

The β form of pro–IL-1 (IL-1β), IL-17, TNFα, and IFN-γ concentrations from the culture supernatant were diluted to the appropriate concentration and measured by commercial ELISA kits (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

Tryptophan metabolites measurement

Levels of tryptophan, 5-hydroxytryptophan, and kynurenine within C. albicans/PBMC coculture supernatant were quantified by UV detection with HPLC. This was performed on a Spectra-System autosampler and pump (Thermo Separation Products, San Jose, CA). Chromatographic separation was performed using an Inertsil 5 ODS-2 column (100 mm × 3.0 inner diameter) (Varian, Middelburg, The Netherlands). Absorbance was monitored with a diode-array detector (UV6000LP; Thermo Separation Products) at a wavelength of 280 nm for tryptophan and 360 nm for kynurenine. The mobile phase for isocratic elution was made by dissolving 40 mM sodium acetate. The pH of the eluent was adjusted to 4.5 with 40 mM sodium acetate. The pH of the eluent was adjusted to 4.5 with 0.3 μl/min (20). For calibration, the standard was diluted in RPMI 1640 in the concentration range of 0.72 μM for tryptophan and 0.42 μM for kynurenine. A total of 50 μl standard or sample was injected into the column for measurement.

Quantitative PCR

PBMCs were stimulated as described above. After 24 h, the supernatant was removed, and the cells were resuspended in 200 μl RNAzolB RNA isolation solvent (Campro Scientific, Veenendaal, The Netherlands) and stored at −80°C. mRNA was isolated according to the instruction of manufacturer. cDNA was synthesized from 1 μg total RNA by use of SuperScript reverse transcriptase (Invitrogen, Carlsbad, CA). Relative mRNA levels were determined using the Bio-Rad i-Cycler (Bio-Rad, Hercules, CA) and the SYBR Green method (Invitrogen). The following primers were used: Foxp3 forward primer 5'-CTGCCCCCTAGTCATGTTG-3' and reverse primer 5'-CTGGGAAGGTGGCTCTTAAAGT-3'; cetinoid acid-related orphan receptor γt (GPR30) forward primer 5'-CCGCTGAGAGGCTTACAC-3' and reverse primer 5'-TGGCCGACACCACCCTCA-3'; GATA-3 forward primer 5'-TACACAAAATGACCGAGACACC-3' and reverse primer 5'-GGTGGGTCTGCGACAGTTCGCAC-3'; T-bet forward primer 5'-CAAGGGGGGCGT-CCAAACATG-3' and reverse primer 5'-TCCGGCTCTTGCTGCATGTA-3'; IDO forward primer 5'-GGTCTGAGGATGTCCTGGA-3' and reverse primer 5'-ACCAATAGAGACAGGAAAGAAGG-3'; and β2-microglobulin forward primer 5'-ATGATGTGCC TGCCGTTG-3' and reverse primer 5'-CCTGAAGG GACCTTCAAAAC-3' (Biolegio, Nijmegen, The Netherlands). Values are expressed as fold increases in mRNA levels relative to those in unstimulated cells.

Statistical analysis

Results from at least three sets of experiments with a minimum of six volunteers were pooled and analyzed using GraphPad Prism software (GraphPad, San Diego, CA). Data are given as mean ± SE, and the Wilcoxon matched pairs test was used to compare differences between groups. The level of significance was set at p < 0.05.

Results

Live C. albicans inhibits heat-killed C. albicans-induced IL-17 production induced by heat-killed C. albicans in human PBMCs

In a previous study (12), we found that heat-killed C. albicans could effectively induce IL-17 production in human PBMCs respectively. Surprisingly, when PBMCs were cocultured with heat-killed and live C. albicans simultaneously, no IL-17 production was detectable from the culture supernatant (Fig. 1A). There are two possible explanations for this unexpected result. Firstly, this might have resulted from the killing of PBMCs due to the outgrowth of live C. albicans in the coculture system. Secondly, live C. albicans may release soluble factors that actively inhibit IL-17 production in PBMCs.

To exclude the first possibility, the concentration of lactase dehydrogenase (LDH) in the supernatant was determined. No significant difference in LDH release was observed, irrespective of whether heat-killed or live C. albicans yeasts were added to PBMCs (Fig. 1B). In addition to LDH measurement, trypan blue staining was used to assess cell viability, and similar results was obtained (data not shown). These results rule out the concern that killing of the PBMCs due to the outgrowth of C. albicans may explain the inhibition of IL-17 production as specific.

Furthermore, IL-1β and TNF production were also determined, and no inhibition of these two cytokines was observed when PBMCs were cocultured with heat-killed and live C. albicans (Fig. 1C), implying that this inhibitory effect is specific for T cell-derived cytokines and especially IL-17 production. A moderate effect on the production of IFN-γ was also observed (Fig. 1C) We further titrated the concentration of heat-killed and live C. albicans in the coculture, and we found that the inhibition of IL-17 is directly proportional to the amount of live C. albicans microorganisms in the culture when heat-killed C. albicans was used at the concentration of 10⁵ and 10⁶/ml (Fig. 1D).

To further dissect the possible mechanism of this inhibitory effect induced by C. albicans, a transwell system was applied to separate live C. albicans from PBMCs. In line with what we observed in the coculturing system, IL-17 induced by heat-killed C. albicans was inhibited when live C. albicans was present in the upper well of the transwell system (Fig. 1E). Similarly, live C. albicans could also inhibit live C. albicans-induced IL-17 production in the transwell system (Fig. 1E). In addition, we also assessed this IL-17 inhibition ability by adding the conditioned medium of the nonpathogenic Saccharomyces cerevisiae to the heat-killed C. albicans and PBMC coculture system. We found that S. cerevisiae-conditioned medium could slightly enhance IL-17 production induced by heat-killed C. albicans (Fig. 1F).

Together, these results imply that certain soluble factors secreted by C. albicans can actively inhibit/suppress IL-17 production induced by both heat-killed and live C. albicans in PBMCs.
The IL-17–inhibiting effect is independent from shedding of pathogen-associated molecular patterns

It is known that some oligomannose and β-glucan could be shed from *C. albicans* cell wall into the culture medium during the culture process. One could hypothesize that instead of shedding the pathogen-associated molecular pattern (PAMPs) passively during the growing process, these shedded PAMPs might play an active role by competing with the heat-killed Candida for the pattern recognition receptor (PRR) binding sites on the PBMCs, thus blocking downstream signaling. To test whether shedding of the PAMPs plays a role in inhibiting IL-17 production, the major PRRs of *C. albicans*, TLR4, TLR2, and dectin-1, were blocked by *Bartonella* LPS, TLR2 antagonist Ab, and laminarin, respectively. However, no difference in IL-17 inhibition was observed by adding any of these PRR antagonists (Fig. 2A). Apart from the aforementioned PRRs, we previously reported that mannose receptor

**FIGURE 1.** Human PBMCs were stimulated with heat-killed and live *C. albicans*, respectively, or in combination for 7 d. Supernatant was collected for IL-17 (A), LDH (B), IL-1β, and TNF measurement (C). D, Human PBMCs were stimulated with different doses of heat-killed *C. albicans* (10⁴, 10⁵, and 10⁶/ml) in the presence of different doses of live *C. albicans* (10³, 10⁴, and 10⁵/ml) for 7 d, then the supernatant was collected, and the IL-17 concentration was determined by ELISA. E, In the transwell system, PBMCs were stimulated with heat-killed *C. albicans* or live *C. albicans* in the lower well and live *C. albicans* on the upper well and culture for 7 d. Supernatant was collected for IL-17 measurement postincubation at 37°C for 7 d. F, PBMCs were stimulated with heat-killed *C. albicans* in the presence/absence of *S. cerevisiae*-conditioned medium for 7 d. Supernatant was collected for IL-17 measurement postincubation at 37°C for 7 d. Mean of three separate experiments. n = 6 volunteers. Values are mean ± SD. *p < 0.05.
Ab, respectively, in the lower well of the transwell system, whereas live IL-17 concentration was measured by ELISA.

Another possibility is that the heat-killed organisms bind to a PRR that is not bound by the live organisms, and the binding to this second receptor alters the response to live organisms. To assess this possibility, several TLR agonists were added together with live C. albicans in PBMC’s culture system. However, IL-17 induced by live C. albicans was not inhibited by all of the TLR agonists tested (Fig. 2C). Together, either blocking the existing PRR pathway or activating the MR pathway failed to restore live C. albicans-induced IL-17 inhibition, implying that the IL-17 inhibition effect is independent from shedding of PAMPs.

Secretd aspartic protease is not responsible for IL-17 inhibiting

Because secreted aspartic proteases (Saps) are identified as an important virulence factor in C. albicans infection (21), they can freely diffuse through the membrane from the upper well to the lower well of the transwell system. Therefore, pepstatin A, an aspartic protease inhibitor, was applied in the system to investigate the involvement of Saps in this IL-17-inhibition effect. Nevertheless, the addition of pepstatin A also failed to reverse the IL-17-inhibiting effect induced by live C. albicans (Fig. 2D), indicating that the enzymatic activity of Saps is not responsible for the downregulation of IL-17 exerted by live C. albicans.

The pattern of Th-specific transcription factors is not influenced by live C. albicans

The next question we asked ourselves is whether this IL-17-inhibition effect resulted from the expression impairment of the Th17-specific RORγt transcription factor. To assess this possibility, we stimulated PBMCs with heat-killed C. albicans in the absence or presence of live C. albicans for 24 h, then total mRNA was isolated, and different transcription factors that are specific to the different Th subtypes were determined by RT-PCR. RORγt mRNA expression as well as T-bet, GATA-3, and Foxp3 expression were not altered by live C. albicans (Fig. 3). Therefore, the inhibition of IL-17 secretion induced by live C. albicans is not mediated by the modulation of the expression patterns of Th-specific transcription factors.

Tryptophan metabolites modulate IL-17 production

It has been demonstrated by Bozza et al. (22) that tryptophan metabolism plays a crucial role in C. albicans infection through modification of antifungal host-defense mechanisms. In view of this, we hypothesized that tryptophan metabolites might be involved in the IL-17 inhibitory effect of live C. albicans.

In mammalian cells, two pathways of tryptophan metabolism have been described. One pathway depends on the enzymatic activity of IDO, which leads to the L-kynurenine synthesis and thereafter to niacin as the end metabolite through a cascade of biochemical reactions. The second pathway is mediated by the modulation of the expression patterns of Th-specific transcription factors.

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FIGURE 2. A. Human PBMCs were stimulated with heat-killed C. albicans together with laminarin, chitin, Bartonella LPS, and anti-TLR2 antagonist Ab, respectively, in the lower well of the transwell system, whereas live C. albicans was present in the upper well. Supernatants were collected after 7 d, and IL-17 concentration was measured by ELISA. B. Human PBMCs were stimulated with heat-killed C. albicans together with live C. albicans mannan in the lower well of the transwell system in the presence of live C. albicans on the upper well. Supernatants were collected after 7 d, and IL-17 concentration was measured by ELISA. C. Human PBMCs were stimulated with live C. albicans together with LPS, Pam3Cys, and mannan, respectively. Supernatants were collected after 7 d, and IL-17 concentration was measured by ELISA. D. Human PBMCs were stimulated with heat-killed C. albicans with/without pepstatin A in the lower well of the transwell in the presence of live C. albicans on the upper well. Supernatants were collected after 7 d, and IL-17 concentration was measured by ELISA. Mean of three separate experiments, n = 6 volunteers. Values are mean ± SD. *p < 0.05.
The next step was to identify the presence of tryptophan metabolites within the supernatant of our experiment. With the help of HPLC analysis, we were able to determine the concentration of tryptophan and tryptophan metabolites within the culture supernatant. We found that tryptophan was consumed by the PBMCs after 7 d of culture without any obvious increase of other tryptophan metabolites, such as L-kynurenine and 5-hydroxytryptophan (Fig. 4B). However, when PBMCs were stimulated with heat-killed C. albicans, significant increase of L-kynurenine concentration was measured in the supernatant without any production of 5-hydroxytryptophan. This implies that upon stimulation of PBMCs with heat-killed C. albicans, tryptophan metabolism was shifted toward more L-kynurenine synthesis. On the contrary, when PBMCs were stimulated with live C. albicans or both live and heat-killed C. albicans, the production of L-kynurenine was lower than the PBMCs with heat-killed C. albicans group, and this was accompanied by an increased 5-hydroxytryptophan concentration (Fig. 4C, 4D).

**FIGURE 3.** Human PBMCs were stimulated with/without heat-killed C. albicans in the lower well of the transwell in the presence/absence of live C. albicans on the upper well. Total mRNA was isolated after 24 h incubation. RORγt, Foxp3, T-bet, and GATA-3 mRNA expression levels were determined by RT-PCR with gene-specific primer pairs. The individual gene expression was normalized to the nonstimulated PBMC control. Mean of three separate experiments. n = 6 volunteers. Values are mean ± SD. *p < 0.05.

**FIGURE 4.** A, Human PBMCs were stimulated with heat-killed C. albicans together with 100 μg/ml tryptophan, 5-hydroxytryptophan, and L-kynurenine, respectively. Supernatant was collected after 7 d, and the IL-17 concentration was determined by ELISA. B–D, Human PBMCs were stimulated with/without heat-killed C. albicans in the lower well of the transwell in the presence of live C. albicans in the upper well. Supernatant was collected after 7 d of culture, then the concentration of tryptophan, 5-hydroxytryptophan, and L-kynurenine was measured by HPLC.
Live C. albicans actively shifts tryptophan metabolism

Because IDO and tryptophan hydroxylase are the key enzymes that determine the direction to be taken by tryptophan metabolism, the mRNA expression of these two enzymes was determined by RT-PCR. As expected, IDO expression was significantly upregulated (~43.3-fold increase) when heat-killed C. albicans was used as the only stimulant. On the contrary, live C. albicans by itself only induced marginal expression of IDO. Moreover, live C. albicans can significantly downregulate IDO expression induced by heat-killed C. albicans up to 67% (from 43.3-fold to 13.9-fold) (Fig. 5A). This result is in agreement with the tryptophan metabolites within the culture supernatant, where we observed that live C. albicans could downregulate L-kyurenine induced by heat-killed C. albicans. In contrast, the expression of tryptophan hydroxylase is constitutive and not influenced by either heat-killed C. albicans or live C. albicans (Fig. 5B).

Following these findings, we further dissected the mechanism through which live C. albicans modulates IDO expression in the host. It is reported that IFN-γ is critical for the induction of IDO expression (23). Therefore, we assessed whether the difference we observed in IDO expression is due to the modulation of IFN-γ production. As expected, IFN-γ production induced by heat-killed C. albicans was significantly reduced by live C. albicans (Fig. 5C). This result further strengthens the conclusion that live C. albicans can actively shift tryptophan metabolism from the L-kyurenine arm to the 5-hydroxytryptophan arm, thus inhibiting host IL-17 production.

Discussion

An increasing body of evidence has demonstrated an important role of IL-17 in host defense against C. albicans infections (3, 24). Nevertheless, most of these studies either focused on the capacity of host immune system in recognizing C. albicans, leading to IL-17 production (25), or were conducted by using IL-17 or IL-17R knockout mice to investigate the role of IL-17 in C. albicans infection in vivo (26). However, these studies did not address the clinical observation that ~30% of healthy individuals at any given moment are colonized with C. albicans, implying that this commensal fungus possesses an armory to modulate the host defense mechanisms responsible for its elimination.

We and others (12, 25) have reported that upon stimulation with C. albicans, PBMCs produce a significant amount of IL-17. In the current study, we show that when PBMCs were cocultured, the IL-17 production was downregulated. One noteworthy finding is that the same concentration of live C. albicans inhibited more strongly IL-17 production induced by a higher inoculum of heat-killed C. albicans. A possible explanation is that in the presence of high inocula of heat-killed C. albicans, PBMCs would preferentially phagocytose heat-killed C. albicans due to their exposure of β-glucan (27). This will compete with the phagocytosis and killing of the live yeasts, leading to higher extracellular growth of live C. albicans and subsequently stronger IL-17 inhibition. Another interesting observation was that with the increased concentration of live C. albicans in the system, the IL-17 produced by PBMCs was also lower. This might be due to either more killing of the PBMCs at the higher innoculum of C. albicans or a pronounced inhibition of IL-17 due to more secreted factors released by live C. albicans. However, a possible decrease in the viability of PBMCs was not the cause of IL-17 inhibition, as shown by normal LDH concentration in the presence of live C. albicans and the normal release of other proinflammatory cytokines, such as TNF and IL-1β.

The biggest hurdle for the study of the in vitro interaction between live microorganisms and host cells is the outgrowth of microorganisms, which might result in massive cell death. In our present study, we found that when live C. albicans was cultured in direct contact with PBMCs at a dose lower than 10^5/ml, most live C. albicans could be phagocytosed and killed by monocytic phagocytes. However, when live C. albicans was cultured with a concentration higher than 10^5/ml, C. albicans could escape phagocytosis and killing of the live yeasts, leading to higher extracellular growth.

**FIGURE 5.** Human PBMCs were stimulated with/without heat-killed C. albicans in the lower well of the transwell in the presence of live C. albicans on the upper well. Supernatant and total mRNA was isolated after 24 h incubation. IDO (A) and tryptophan hydroxylase (B) mRNA expression level were determined by RT-PCR with gene-specific primer pairs. The individual gene expression was normalized to the nonstimulated PBMC control. C. The IFN-γ concentration in the supernatant after 7 d incubation measured by ELISA. Mean of three separate experiments. n = 5 volunteers. Values are mean ± SD. *p < 0.05.
within the culture well, accompanied by increasing cell death through trypan blue staining. To bypass this hurdle, we have successfully adopted a transwell system to avoid the direct contact between live *C. albicans* and PBMCs and demonstrated that soluble factors released by *C. albicans* actively modulated cytokine profiles induced by heat-killed *C. albicans* in PBMCs within 24 h of coculturing (19).

The role of several potential mechanisms responsible for this inhibitory effect has been assessed. Neither the release of *C. albicans* PAMPs nor the enzymatic activity of secreted aspartic acid or the altering of the differentiation of Th cell subsets was responsible for this IL-17–dampening effect. Therefore, we investigated whether tryptophan metabolism was altered by live *C. albicans*, as tryptophan metabolites have been reported to be involved in Th17 responses (29). Surprisingly, by adding different tryptophan metabolites to the *C. albicans*-stimulated PBMCs, the presence of 5-hydroxytryptophan but not L-kyurenine resulted in the inhibition of IL-17 production. In line with this finding, there was no detectable 5-hydroxytryptophan in the supernatant from heat-killed *C. albicans* cocultured with PBMCs, yet a higher concentration of 5-hydroxytryptophan was determined from PBMCs incubated with live *C. albicans*. Moreover, we also found that live *C. albicans* could inhibit IFN-γ production induced by heat-killed *C. albicans* and therefore leads to lower IDO mRNA expression. In contrast, tryptophan hydroxylase expression remains unchanged. Subsequently, the tryptophan metabolism was shifted toward 5-hydroxytryptophan production, and the IL-17 production was downregulated.

Bozza et al. (22) previously reported that when mice were infected with *C. albicans*, an increased IDO expression and higher kyurenine secretion was detected at the site of *C. albicans* infection. They also demonstrated that if IDO activity was impaired by 1-methyl-tryptophan treatment, kyurenine level was also reduced and at the same time drastically impaired resistance to infection. Our current finding of the role of 5-hydroxytryptophan in inhibiting IL-17 production further explains how the inhibition of IDO, shifting tryptophan metabolism toward 5-hydroxytryptophan, could lead to an impaired resistance to infection, based on the fact that IL-17 was critical for anti-*C. albicans* host defense.

Tryptophan metabolism is involved in the modulation of several immune responses, ranging from antimicrobial activity by tryptophan starvation (30), protection of allogeneic fetus (31), amelioration of autoimmune diseases (32, 33), tumor resistance (34, 35), and chronic granulomatous disease (29). Among the tryptophan metabolic pathways, IDO is one of the best-characterized enzymes in terms of immunological effects (36, 37), and many experiments were conducted to investigate the functionality of IDO in immune regulation by blocking its enzymatic activity with 1-methyl-tryptophan. However, the interpretation of the results by simply emphasizing the function of IDO might be risky, because there could be either a compensation effect by another redundant enzymatic function or the production of different tryptophan metabolites by other homeostatic enzymes. In line with that, we observed that the expression of tryptophan hydroxylase was not changed, yet IDO expression was significantly reduced by live *C. albicans*, leading to a shift of the final tryptophan metabolites and inhibition of downstream IL-17 production. Therefore, our novel finding of the role of 5-hydroxytryptophan for modulation of *C. albicans*-induced IL-17 production sheds new light on the modulatory effects of IDO, and it is tempting to speculate that 5-hydroxytryptophan or other tryptophan metabolites might play a role in IDO-mediated immune regulation.

In conclusion, our findings have several important immunological and clinical consequences. Firstly, we demonstrate for the first time that a pathogen can actively shift the balance of tryptophan metabolism in the host, with immunomodulatory effects on the host defense. Secondly, this has direct implications for the way in which a *C. albicans* infection is handled. In an immunocompetent host, rapid phagocytosis and intracellular killing of *C. albicans* prevents the shift of the tryptophan metabolites and the inhibition of IL-17, with effective infection resolution. In contrast, persistence of *C. albicans* in an immunosuppressed individual would permit growth, induction of the 5-hydroxytryptophan pathway, and further dampening of host defense by IL-17 inhibition. Moreover, one could hypothesize that IL-17 inhibition may also play a role in the colonization of mucosal surfaces by *C. albicans*.

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Disclosures

The authors have no financial conflicts of interest.

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