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Candida albicans THI13 disruption affects production of monocytic cytokines

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Introduction: The *Candida albicans THI13* gene was identified by its homology to the *Candida tropicalis CtNMT1* gene, which is involved in pyrimidine precursor biosynthesis.
Methods: Disruption of *THI13* revealed that this gene played a minor role in thiamin biosynthesis in *C. albicans*. Purified human monocytes were incubated with *C. albicans* at the optimal *Candida*: monocyte ratio of 0.5 and cytokines in the supernatants were measured by enzyme-linked immunosorbent assay.
Results and discussion: This experiment showed that the wild-type strain significantly induced interleukin-10 (IL-10) production but had little effect on IL-12 production, and that *THI13* mutants had no significant effect on IL-10 production, though the IL-12 level was increased in the supernatants. These results suggest that THI13 is involved in the host effective immune response by regulating IL-10 and IL-12 production.

Key words: *Candida albicans*; THI13; interleukin-10; interleukin-12

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Candida albicans is the most prevalent human opportunistic fungal pathogen and may cause various forms of candidiasis ranging from superficial mucosal infections to life-threatening systemic diseases (7, 18). Most candidiases, for example oral candidiasis, have been consistently associated with defects in the immune response so the capacity to control disease lies, in part, in the ability of the host to generate an appropriate immune response. Monocytes are involved in both cellular and humoral immune responses and serve as important effector cells of both innate and specific immunity during infectious diseases, including candidiasis (3, 15). Cytokines, especially the immunoregulatory cytokines interleukin-10 (IL-10) and IL-12, are released from monocytes interacting with *Candida* organisms (19). Characteristically, most pathogenic *C. albicans* have the ability to create an environment that is rich in IL-10 and poor in IL-12, and this unbalanced IL-12 versus IL-10 induction may lead to increased pathogenicity and evasion of the host protective immunity.

Thiamin diphosphate is a co-enzyme of the sugar metabolism system. The yeast *Saccharomyces cerevisiae* utilizes external thiamin for the production of thiamin diphosphate or can synthesize the cofactor itself (1, 10). Several genes involved in thiamin biosynthesis have been identified, including the *S. cerevisiae THI5* gene family (*THI5*, *THI11*, *THI12* and *THI13*), the *Schizosaccharomyces pombe NMT1* gene and the *CtNMT1* gene of *Candida tropicalis* (11, 13). Interestingly, a *CtNMT1* disruptant did not show thiamin auxotrophy but produced pseudohyphal filaments, although exogenous thiamin repressed its expression.

The distinct morphological forms of *C. albicans* are known to elicit different immune responses by human hosts. Additionally, previous studies have shown that the *C. tropicalis* gene (*CtNMT1*), believed to be involved in thiamin biosynthesis, can also influence morphology. In the present study a homologue of the *CtNMT1* gene of *C. tropicalis* was identified in *C. albicans* and its effect on the production of monocytic cytokines was determined.

Materials and methods

Strains and media

The *C. albicans* strains used for this study are listed in Table 1. The yeast media used were YPD (1% yeast extract, 2% peptone, 2% glucose), minimal medium [0·67% (weight/volume) Difco yeast nitrogen base without amino acids, 2% glucose] and Wickerham's medium. Where indicated, thiamin was added to a final concentration of 2 μM. Synthetic Defined (SD) medium

Table 1. Candida albicans strains and plasmids used

	Parent	Genotype
Strains		
CAF2–1	SC5314	*ura3*Δ::*imm434/URA3*
CAI4	CAF2–1	*ura3*Δ::*imm434/ura3*Δ::*imm434*
C125K1	CAI4	*THI13/thi13*Δ::*hisG-URA3-hisG*
C125K1a	C125K1	*THI13/thi13*Δ::*hisG*
C125K2	C125K1a	*thi13*Δ::*hisG/thi13*Δ::*hisG-URA3-hisG*
C125K2a	C125K2	*thi13*Δ::*hisG/thi13*Δ::*hisG*
C125EXP	C125K2a	*thi13*Δ::*hisG/thi13*Δ::*hisG*::*THI13*
Plasmids		
p5921		*hisG-URA3-hisG cassette*
p125		*Full-length THI13 gene*
p125K		*hisG-URA3-hisG inserted into THI13 gene*
pCaEXP		*Expression vector*
pCaEXP-THI13		*Expression vector containing THI3 gene*

was supplemented with a complete synthetic mix containing all the amino acids and bases. For prototrophic selection of yeast, the powder mix was made up as before, omitting the relevant nutrient. *Escherichia coli* strain DH5α and Luria-Bertani (LB) medium were used for transformation and plasmid DNA preparation.

Identification of C. albicans THI13

To improve our understanding of the gene function of *C. albicans*, we had previously constructed a complementary DNA (cDNA) library (20). Briefly, Total RNA was isolated from *C. albicans* SC5314 treated with different drugs and harvested at different growth phases. The messenger RNAs were subsequently purified and used as the template for cDNA synthesis. The cDNA library was constructed in PBSF, a modified pBluescript II SK vector (Stratagene, La Jolla, CA), by using the SMART (switching mechanism at 5′ end of the RNA transcript) cDNA library construction kit (catalog no. K1051–1; Clontech, Palo Alto, CA) according to the manufacturer's instructions. The nucleotide sequences of *CtNMT1* in *C. tropicalis* were used to search the National Center for Biotechnology Information (http://www.ncbi.nih.gov/BLAST/), the Stanford sequence database (http://www.sequence.stanford.edu/group/candida/) and the CandidaDB database (http://genolist.pasteur.fr/CandidaDB/). The *C. albicans* homologue was used to search the nucleotide database of our self-constructed *C. albicans* cDNA library and the corresponding clones were sequenced. Multiple alignments of deduced amino acid were performed with CLUSTAL W version 1.82 (http://www.ebi.ac.uk/clustalw/).

Northern blot analysis

Cells were grown for 24 h in Wickerham's medium supplemented with 2 μM thiamin or without thiamin. Total RNA was prepared as described previously (2) and ^{32}P-labeled DNA probes were generated by random priming using the THI13 cDNA fragment as the template. ACT1 was used as a loading control.

Disruption of *THI13* in *C. albicans*

To investigate the function of *THI13* in *C. albicans*, the gene was disrupted by the method described by Fonzi and Irwin (6). The hisG-URA3-hisG cassette was derived from plasmid p5921 by *Bgl*II and *Bam*HI digestion and inserted into the *Bam*HI site of p125, which was the plasmid containing the full sequence of *THI13* from our cDNA library, thus producing p125K. The p125K was linearized by *Pvu*II and used to transform *C. albicans* strain CAI4. The *C. albicans* strains, heterozygous for the first *THI13* disrupted allele, were further utilized for the disruption of the second remaining wild-type allele. The verification of *THI13* disruption in heterozygous and homozygous strains was monitored by polymerase chain reaction and Southern blotting using a random-primed digoxigenin DNA-labeling detection kit (Roche, Mannheim, Germany). Chromosomal DNA isolated from all strains was digested with *Pst*I. A 681-base-pair *Hpa*I–*Bam*HI DNA fragment derived from plasmid p125 was used as a specific probe for Southern analysis. The wild-type *THI13* allele showed a 5.7-kilobase (kb) band. A new 9.74-kb band, which represented the Ura blaster integrated into one *THI13* allele, appeared in the transformants. After selection on medium containing 5-fluoroorotic acid, the loss of the *URA3* gene and one copy of the *hisG* element resulted in a 6.85-kb band. The thi13::*hisG*/Δ*thi13*::*hisG-URA3-hisG* strains showed 6.85-kb and 9.74-kb bands while the corresponding Ura-negative derivatives showed a 6.85-kb band (Fig. 1).

For reversion of homozygous *thi131*Δ strains, the whole *THI13* gene was cloned into the integrative *C. albicans* expression vector pCaExp (16) with primer A (5′-gcgctgcagcaaaagaggaggaatttgaatat-3′) and primer B (5′-cggctgcagatacatatctttgtttcttactaat-3′). This vector carries the *URA3* gene under its native promoter and was designed for integration into the *RPS10* locus. The recombinant plasmid pCaEXP-THI13 was linearized and used to transform c125K2a. Strains expressing the *URA3* gene at the RPS10 locus were constructed by transforming the linearized pCaEXP plasmid to c125K2a to integrate the plasmid at the target locus.

Examination of the role of *THI13* in thiamin biosynthesis

Cells were grown overnight in liquid YPD medium, washed twice with water and adjusted to approximately 1×10^8 cells/ml. Aliquots (5 μl) of one-tenth serial dilutions beginning with 1×10^8 cells/ml were spotted onto Wickerham's medium supplemented with 2 μM thiamin or without thiamin and incubated for 7 days at 30°C. The growth of the strains was then compared.

Coculture of C. albicans with monocytes

Peripheral blood mononuclear cells were isolated from heparinized blood of healthy volunteers by Histopaque-1077 (Sigma Chemical Company, St Louis, MO) gradient centrifugation. For isolation of monocytes, the peripheral blood mononuclear cell-enriched layer was treated with the magnetic antibody cell sorter (MACS) Monocyte Isolation Kit (Miltenyi Biotec, Auburn, CA). One colony of *C. albicans* from fresh YPD agar plates was transferred to YPD liquid medium and incubated overnight in an orbital shaker at 30°C. Cells were harvested, washed with sterile phosphate-buffered saline and counted in a hemocytometer. Monocytes (2×10^6 cells) were incubated in RPMI-1640 plus 5% inactivated fetal calf serum at 37°C in 5% CO_2 for 2 h. *C. albicans* was added to the monocytes at various yeast: monocyte ratios and incubated for a further 20 h. Supernatants were then collected and stored at −70°C until use. The viability of monocytes in co-culture with live *C. albicans* was monitored using a LIVE/DEAD Viability/Cytotoxicity kit (Molecular Probes, Inc., Eugene, OR). The yeast: monocyte

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C. albicans     MSTNKITFLLNWEAAPYHIPVYLANIKGYFKDENLDIAILEPSNPSDVTELVGSGKVDMG 60
C. tropicalis   MSTDKITFLLNWEVAPYHIPVYLASQKGYFKEEGIDVAILEPSNPSDVTELIGSGKVDMG 60
S. cerevisiae   MSTDKITFLLNWQPTPYHIPIFLAQTKGYFKEQGLDIAILEPTNPSDVTELIGSGKVDMG 60
                ***:********: :******::**. ******:::*:*:*****:********:********

C. albicans     LKAMVHTLAAKARGLPVTSIGSLLDEPFTGICYLEGSGITSDFQSLKGKRIGYVGEFGKI 120
C. tropicalis   LKAMVHTLAAKARGYPVTSIGSLLDEPFTGICYLEGSGITSDFQSLKGKRIGYVGEFGKI 120
S. cerevisiae   LKAMIHTLAAKARGFPVTSVASLLDEPFTGVLYLKGSGITEDFQSLKGKKIGYVGEFGKI 120
                ****:********* ****:.*********: **:*****.********:**********

C. albicans     QVDELTKHYGMTPDDYVAVRCGMNVAKYILEGTIDCGIGIECIQQVELEEALKEQGKDSN 180
C. tropicalis   QVDELTKHYGMTPDDYTAVRCGMNVAKYILEGQIDCGIGIECIQQVELEEALKKQGKDPN 180
S. cerevisiae   QIDELTKHYGMKPEDYTAVRCGMNVAKYIIEGKIDAGIGIECMQQVELEEYLAKQGRPAS 180
                *:*********.*:**.************:** **.******:******* * :**: ..

C. albicans     DAKMLRIDKLAELGCCCFCTILYIANDKFIAENSQAVKKFLKAIKRATDYMLAHPREAWA 240
C. tropicalis   GAKMLRIDKLAELGCCCFCTILYIANDKFIAENPDKIKKFLKAVKKATDYMLANPKQAWA 240
S. cerevisiae   DAKMLRIDKLACLGCCCFCTVLYICNDEFLKKNPEKVRKFLKAIKKATDYVLADPVKAWK 240
                .********** ********:***.**:*: :*.: ::*****:*:****:**.* :**

C. albicans     EYGNFKPTMQTDLNTKKFQRCYAYFSESLYNVHRDWRKVNNYGKRLDILPENYVPNYTNE 300
C. tropicalis   EYGNFKPNMQSELNTKKFSRCFAYFSDSLYNVHRDWRKVNNYGKRLEILPADYVPNYTNE 300
S. cerevisiae   EYIDFKPQLNNDLSYKQYQRCYAYFSSSLYNVHRDWKKVTGYGKRLAILPPDYVSNYTNE 300
                ** :*** ::.:*. *::.**:****.*********:**..***** *** :**.*****

C. albicans     YLSWPEPKEVDDPEKAQDLMLKHQEECKTCGGYKRLVLA-- 339
C. tropicalis   YLSWPEPQEVSDPLKAQELMAKHQEECKTCGGYKRLVLSGI 341
S. cerevisiae   YLSWPEPEEVSDPLEAQRLMAIHQEKCRQEGTFKRLALPA- 340
                *******:**.** :** **  ***:*:  * :***.*.
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Fig. 1. Sequence alignment of the deduced amino acid sequence of *Candida. albicans THI13* with *Candida. tropicalis Ctnmt1p* and *Saccharomyces. cerevisiae Thi13p*. An asterisk indicates that residues in that column are identical in all sequences; a colon indicates that conserved substitutions are observed; and a point indicates that semi-conserved substitutions are observed.

ratios examined were 0.01, 0.05, 0.5, 1, 10 and 50.

Cytokine quantification by enzyme-linked immunosorbent assay

Cytokine proteins in the cell supernatants were quantified by enzyme-linked immunosorbent assay (ELISA) with IL-10 and IL-12 ELISA kits (Bender Med Systems, Vienna, Austria) The ELISA procedure was performed according to the manufacturer's instructions. The concentrations of the cytokines were determined with reference to a standard curve for serial twofold dilutions of the cytokines provided by the kit. The optical absorption was measured at 450 nm and the sensitivity of the ELISA was >1 pg/ml.

Statistics

Results were expressed as mean ± standard error for n number of repeat experiments. Statistical significance was determined by Student's t-test. $P < 0.05$ was considered significant.

Results

Homology study of *THI13*

Comparison of databases showed that the cDNA fragment of the *C. albicans* homologue from our *C. albicans* cDNA library had 99% of identity with Ca019.7324, containing an open reading frame 1017 base pairs long and encoding a 339-amino-acid protein. This protein had 73% and 87% identity with Thi13p of *S. cerevisiae* and Ctnmt1p of *C. tropicalis*, respectively (Fig. 2). By homology, this protein has been named Thi13p and it is predicted to

be involved in the biosynthesis of one of the thiamin precursor molecules, pyrimidine precursor biosynthesis, with a calculated molecular mass of 38.3 kDa. Chromosomal mapping of *THI13* revealed its presence on chromosome R of the *C. albicans* genome.

Role of *THI13* in thiamin biosynthesis

The growth of the wild-type strain CAF2–1 and the thi13-revertant strain c125EXP, in which *THI13* gene was intact, was stronger than that of the strains grown in Wickerham's medium lacking thiamin, while the growth of the thi13 mutants c125K1 and c125K2 was slow, which was indicative of thiamin auxotrophy (Fig. 3A). Addition of thiamin to the Wickerham's medium resulted in the growth of thi13 mutants comparable with that of the wild-type and revertant strains (data not shown). Northern blot showed that *THI13* gene expression in CAF2–1 grown in Wickerham's medium without thiamin was significantly higher than in CAF2–1 grown in Wickerham's medium supplemented with thiamin (Fig. 3B).

IL-10 and IL-12 induction

To rule out the killing of monocytes by *C. albicans* during co-culture, the viability of monocytes exposed to *C. albicans* was determined first. The results showed that

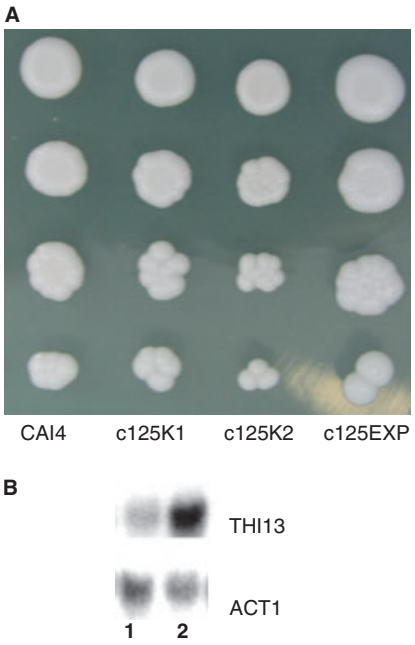

Fig. 3. (A) Phenotypic analysis of thi13 mutants grown on Wickerham's medium lacking thiamin. Cells were grown overnight in liquid YPD medium, washed with water and adjusted to approximately 1×10^8 cells/ml. Aliquots (5 µl) of 1/10 serial dilutions beginning with 1×10^8 cells/ml were spotted onto Wickerham's medium lacking thiamin and incubated for 7 days at 30°C. (B) Northern blot analysis of *THI13* expression in CAF2–1 grown for 24 h in Wickerham's media supplemented with 2 µM thiamin (lane 1) or without thiamin (lane 2). ACT1 was used as a loading control.

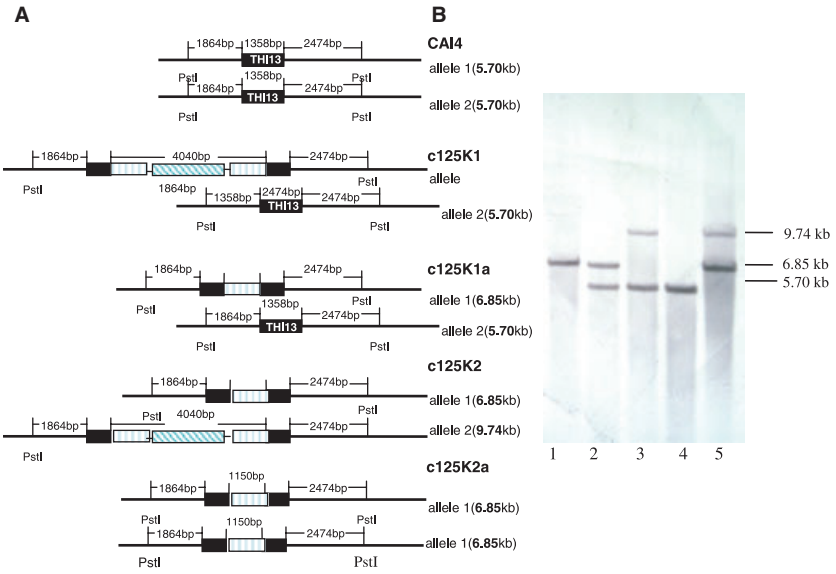

Fig. 2. Disruption of *THI13* in *Candida. albicans*. (A) Genetic organization of the *THI13* locus in the strains. The position and size of *THI13* (black bar), *URA3* (slash bar) and *HisG* (erected bar) are shown. (B) Southern analysis of genomic DNA digested with *Pst*I. Lane 1, c125K2a (*ura3*Δ/*ura3*Δ, thi13Δ::*hisG*/thi13Δ::*hisG*); lane 2, c125K1a (*ura3*Δ/*ura3*Δ, *THI13*/thi13Δ::*hisG*); lane 3, c125K1 (*ura3*Δ/*ura3*Δ, *THI13/thi13*Δ::*hisG-URA3-hisG*); lane 4, CAI4 (*ura*Δ/*ura3*Δ, THI13/THI13); lane 5, c125K2 (*ura3*Δ/*ura3*Δ, *thi13*Δ::*hisG-URA3-hisG*/*thi13*Δ::*hisG*).

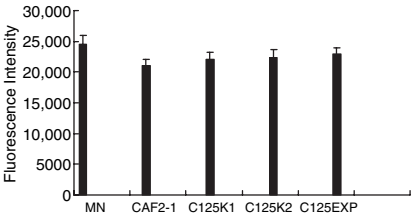

Fig. 4. Viability of monocytes co-cultured with *Candida. albicans* at a 0.5 ratio of *C. albicans*: monocytes (MN). Monocytes were co-cultured with CAF2–1, c125K1, c125K2 or c125EXP. The fluorescence intensity represents monocyte viability as determined by cytofluorometry. The data shown represent three independent experiments with similar results.

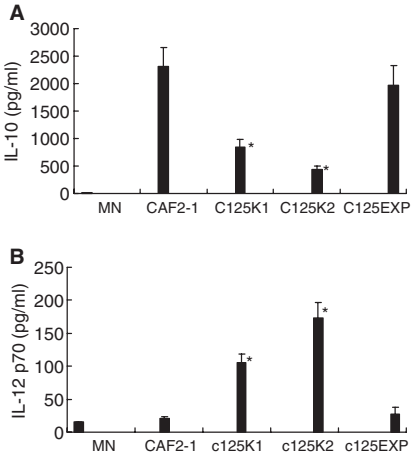

Fig. 5. Candida. albicans differentially induces IL-10 (A) and IL-12 p70 (B) production by monocytes (MN). Monocytes were treated with CAF2–1, c125K1, c125K2 or c125EXP and supernatants were collected after incubation for 20 h. Cytokines were measured by enzyme-linked immunosorbent assay. The data shown represent three independent experiments with similar results. Each experiment was performed with cells from eight different blood donors. Statistically significant differences between the wild type (CAF2–1) and thi13 mutants groups were determined by Student's *t*-test: $*P < 0.01$.

there was no statistically significant difference in fluorescence intensity between the tested groups at a yeast: monocytes ratio of 0.5 (Fig. 4). The ratio of 0.5 was therefore chosen to determine the production of immunoregulatory cytokines by monocytes following stimulation by *C. albicans* in all subsequent experiments. It was found that the wild-type and thi13-revertant strains, not the thi13 mutant, induced IL-10 production significantly (both >2000 pg/ml) 20 h after incubation with monocytes; only the thi13 mutant strains strongly induced IL-12 production (both were higher than 100 pg/ml; Fig. 5).

Discussion

Thiamin, which mainly occurs as a diphosphate in the living human body, works as a co-enzyme in the sugar metabolism system (active vitamin B1). Thiamin deficiency causes many clinically significant problems such as dysphoria, quadriplegia and dyspepsia. The present study reports the function of *THI13*, the first gene involved in thiamin biosynthesis in *C. albicans*. Similar to its homologue in *S. cerevisiae*, there was a minor difference in growth rate on the medium lacking thiamin between the wild-type strain and the thi13 mutants (11), indicating that thiamin biosynthesis in *C. albicans* is functionally redundant and some other genes may play roles in this pathway. In *C. tropicalis*, Takahito reported that disruption of *CtNMT1*, a homologue of *S. cerevisiae THI5*, did not result in thiamin auxotrophy but produced pseudohyphal filaments, a new function foreign to thiamin biosynthesis (13). To investigate the function of *THI13* in *C. albicans*, we disrupted both alleles of this gene and examined the phenotypes of the mutants. Interestingly, our results showed that *C. albicans THI13*, in addition to its primary function, affected immunoregulatory cytokine production by monocytes.

Monocytes/macrophages serve as important effector cells of innate and specific immunity during infectious diseases. In addition to their involvement in the elimination of pathogens from the bloodstream and tissues, monocytes/macrophages can produce chemokines and cytokines, which serve to further promote specific immune responses. Cytokines, especially the immunoregulatory cytokines IL-10 and IL-12, are released from human monocytes/macrophages interacting with *Candida* organisms (5, 15). These cytokines may regulate the acquired immune response through T-cell development (12). It was considered that the virulence of *C. albicans* might be related to its ability to induce IL-10, with a selective inhibition of IL-12 production (4). IL-12 exhibits a number of bioactivities that may modulate infectious disease progression, including enhancing natural killer and T-cell cytotoxicity, modulating T-cell proliferation, and potentiating T helper type 1 differentiation. IL-10 is antagonistic on the activity of monocytes against *Candida*. In the murine models of candidiasis, neutralization of IL-10 upregulates nitric oxide production and protects susceptible mice from challenge with *C. albicans*. Our experiments showed that disruption of *THI13* resulted in the decreased production of IL-10 and increased production of IL-12 by monocytes compared with the wild-type strain, suggesting that thi13 mutants might increase the protective type I responses of the host.

It should be noted that the germination of thi13 mutants was not affected in the co-culture with monocytes (data not shown). This phenomenon was in contrast with several published results (4, 19), in which the role of *C. albicans* morphogenesis in the production of cytokines was emphasized and the hyphal forms failed to induce IL-12 while the yeast form suppressed IL-10 but induced IL-12 production. In addition, their results showed that phagocytosis of yeasts by monocytes might cause IL-12 production. Interestingly, Tang et al. reported a different result in their study, where candidal IL-12 production by monocytes was not related to phagocytosis of blastospores (14). Since the yeast form of *C. albicans* is easily phagocytosed by monocytes while the hyphal form is not (17), it is unlikely that yeast is the only form to induce IL-12 production, as shown in our experiments. Factors affecting monocytic IL-12 production include direct cell-to-cell contact (8, 9), interactions through a *C. albicans*-secreted glycoprotein and other unknown mechanisms. An understanding of how a thi13 mutant with normal hyphae is involved in altering monocytic cytokine production may stimulate the development of novel immunorelated therapies for the treatment of candidiasis.

Acknowledgments

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References

1. Begley TP. The biosynthesis and degradation of thiamin (vitamin B_1). Nat Prod Rep 1996: **13**: 177–185.
2. Cao YY, Cao YB, Xu Zh et al. cDNA microarray analysis of differential gene expression in *Candida albicans* biofilm exposed to farnesol. Antimicrob Agents Chemother 2005: **49**: 584–589.
3. Castro M, Bjoraker JA, Rohrbach MS, Limper AH. *Candida albicans* induces the release of inflammatory mediators from human peripheral blood monocytes. Inflammation 1996: **20**: 107–122.
4. Chiani P, Bromuro C, Torosantucci A. Defective induction of interleukin-12 in human monocytes by germ-tube forms of *Candida albicans*. Infect Immun 2000: **68**: 5628–5634.
5. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, De Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991: **174**: 1209–1220.
6. Fonzi WA, Irwin MY. Isogenic strain construction and gene mapping in *Candida albicans*. Genetics 1993: **134**: 717–728.
7. Fridkin SK, Jarvis WR. Epidemiology of nosocomial fungal infections. Clin Microbiol Rev 1996: **9**: 499–511
8. Ghannoum MA. Potential role of phospholipases in virulence and fungal pathogenesis. Clin Microbiol Rev 2000: **13**: 122–143.
9. Grunvald E, Chiaramonte M, Hieny S et al. Biochemical characterization and protein kinase C dependency of monokine-inducing activities of *Toxoplasma gondii*. Infect Immun 1996: **64**: 2010–2018.
10. Hohmann S, Meacock PA. Thiamin metabolism and thiamine diphosphate-dependent enzymes in the yeast *Saccharomyces cerevisiae*: genetics and regulation. Biochim Biophys Acta 1998: **1385**: 201–219.
11. Raymond W, Peter A, Meacock PA, Wightman R. The THI5 gene family of *Saccharomyces cerevisiae*: distribution of homologues among the hemiascomycetes and functional redundancy in the aerobic biosynthesis of thiamin from pyridoxine. Microbiology 2003: **149**: 1447–1460.
12. Romani L, Puccetti P, Bistoni F. Biological role of Th cell subsets in candidiasis. Chem Immunol 1996: **63**: 115–137.
13. Suzuki T. Subtractive gene cloning and gene-disruption for elucidation of pseudohyphal formation in *Candida tropicalis*. Jpn J Med Mycol 2003: **44**: 93–100.
14. Tang Nf, Liu LM, Kang KF et al. Inhibition of monocytic interleukin-12 production by *Candida albicans* via selective activation of ERK mitogen-activated protein kinase. Infect Immun 2004: **72**: 2513–2520.
15. Trinchieri G. Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. Annu Rev Immunol 1995: **13**: 251–276.
16. Umeyama T, Nagai Y, Niimi M, Uehara Y. Construction of FLAG tagging vectors for *Candida albicans*. Yeast 2002: **19**: 611–618.
17. Vazques-Torres A, Balish E. Macrophages in resistance to candidiasis. Microbiol Mol Biol Rev 1997: **61**: 70–192.
18. Wenzel RP. Nosocomial candidemia: risk factors and attributable mortality. Clin Infect Dis 1995: **20**: 1531–1534.
19. Xiong J, Kang K, Liu L, Yoshida Y, Cooper KD, Ghannoum MA. *Candida albicans* and *Candida krusei* differentially induce human blood mononuclear cell interleukin-12 and gamma interferon production. Infect Immun 2000: **68**: 2464–2469.
20. Xu Z, Cao YB, Zhang JD et al. cDNA array analysis of the differential expression change in virulence-related genes during the development of resistance in *Candida albicans*. Acta Biochim Biophys Sin 2005: **37**: 463–472.