

Increased Levels of *Candida albicans* Mannan-Specific T-Cell-Derived Antigen Binding Molecules in Patients with Invasive Candidiasis

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In addition to cytokines, CD4⁺ T cells have been found to secrete soluble, T-cell-derived antigen binding molecules (TABMs). These antigen-specific immunoproteins are thought to have immunoregulatory properties in the suppression of cell-mediated immunity (CMI) because they often associate with interleukin-10 (IL-10) and transforming growth factor beta. Decreased CMI causes susceptibility to infections caused by organisms which are normally nonpathogenic. In this situation, e.g., *Candida albicans* saprophytism may develop into invasive candidiasis. The difficult diagnosis of invasive candidiasis is based on the findings obtained from blood cultures and with tissue biopsy specimens, with some additional diagnostic value gained by the detection of *Candida albicans* mannan antigenemia and antimannan antibodies. In the present study, *Candida albicans* mannan-specific TABM (CAM-TABM) levels in the sera of patients with invasive candidiasis ($n = 11$), *Candida* colonization ($n = 11$) and noncolonization ($n = 10$), recurrent vulvovaginal candidiasis ($n = 30$), and atopic eczema dermatitis syndrome ($n = 59$) and healthy controls ($n = 30$) were analyzed. For 14 participants, the effect of mannan stimulation on TABM production and gamma interferon (IFN- γ) and IL-4 mRNA expression by peripheral blood lymphocytes was also studied. It was demonstrated that CAM-TABM production was the highest in patients with invasive candidiasis and that CAM-TABM levels could distinguish *Candida*-colonized patients from noncolonized patients. In addition, the CAM-TABM level was directly related to mRNA expression for IL-4 but not IFN- γ . These results reinforce the view that TABMs are associated with decreased CMI, immunoregulation, and the T-helper cell 2-type immune response.

Based on their cytokine production, CD4⁺ T-helper (Th) lymphocytes can be divided into Th1 and Th2 cells, with the former producing gamma interferon (IFN- γ), interleukin-2 (IL-2), and tumor necrosis factor beta and with the latter producing IL-4, IL-5, IL-10, and IL-13 (1, 57). The presentation of microbial antigens by dendritic cells (DCs) and macrophages to CD4⁺ T cells and the Th1-type cytokine production by these T cells are essential in cell-mediated immunity (CMI) (1). Decreased CMI gives rise to infections of normally nonpathogenic organisms, such as *Candida albicans*. A proper Th1-type response has proved crucial in the immune defense against *C. albicans* infections, whereas a Th2-type response predisposes the patient to invasive growth and recurrent or chronic infections (37). In a Th2-type immune response, T cells have been found to secrete, in addition to cytokines, soluble, T-cell receptor (TCR) α -chain-related, antigen-specific immunoproteins called T-cell-derived antigen binding molecules (TABMs), which recognize unprocessed antigens independently of major histocompatibility complex class I or II antigens (40, 50, 51). TABMs are thought to participate in the suppression of CMI by an immunoregulatory mechanism, e.g., by an antigen-specific focusing of IL-10 and transforming growth factor beta (TGF- β), because they often associate with these cytokines (4, 5, 26, 40). Furthermore, TABM- and TCR α -chain-related immunoprotein-induced delayed-type hyper-

sensitivity suppression has been shown in anterior chamber-associated immune deviation of the eye, which is an in vivo example of the function of these proteins (11, 55).

Elevated, antigen-specific serum TABM levels have been detected in several diseases. These include *C. albicans* mannan-TABMs (CAM-TABMs) in recurrent vulvovaginal candidiasis (RVVC) and inflammatory bowel syndrome (24, 25); casein-, β -lactoglobulin- and α -lactalbumin-specific TABMs in milk intolerance (26); benzoic acid-TABMs in toluene sensitivity (18); and filarial extract-TABMs in chronic filariasis (24). All these TABMs were shown to carry the same epitope (3C9) detected by a monoclonal anti-TABM antibody, MG3C9-1A12 (24).

TABMs specific for Cetavlon-purified mannan polysaccharide of *C. albicans* (CAM-TABMs) were characterized by Little et al. (25). They proved cross-reactive because they also bound to *C. albicans* whole extract and other fungi, including *Malassezia*. In polyacrylamide gel electrophoresis, reduced, alkylated CAM-TABMs demonstrated 86-, 43-, and 22-kDa protein bands. Furthermore, CAM-TABMs did not contain immunoglobulins, whereas it was noticed that TGF- β 2 was associated with CAM-TABMs.

The diagnosis of invasive candidiasis is difficult and is based on the findings of studies of blood cultures and with tissue biopsy specimens (15). *Candida albicans* mannan has been used as an additional diagnostic tool with some value. Detection of mannan antigenemia (7, 56) or antimannan antibodies (10, 44, 54), or both (46), has enhanced the ability to diagnose candidiasis, especially in blood culture-negative cases.

The purpose of the present study was to demonstrate that *C.*

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TABLE 1. The study subjects

Study group (<i>n</i>)	Age (yr) ^a	No. of patients by sex ^b	Inclusion criteria	Exclusion criterion
Invasive candidiasis patients (11)	51 (33–87)	9 M, 2 F	Any of the following: (i) <i>Candida</i> -positive histology or culture from deep organ at autopsy; (ii) more than one <i>Candida</i> -positive blood culture and clinical signs of septicemia; (iii) <i>Candida</i> -positive culture of sample from a normally sterile body site (other than urine); (iv) <i>Candida</i> -positive culture from central catheter tip or from cutaneous lesions and clinical signs of septicemia; (v) <i>Candida</i> endophthalmitis by fundoscopy and clinical signs of septicemia	Patients with <i>Candida</i> -positive urine (difficult to interpret the severity of infection)
<i>Candida</i> -colonized patients (11)	50 (25–78)	7 M, 4 F	Hospitalization for over a week after operation; <i>C. albicans</i> isolated from clinical specimens other than blood, visceral organs, deep abscess, or urine; no signs of septicemia	
Hospitalized, noncolonized controls undergoing gastrointestinal surgery (10)	58.5 (45–71)	7 M, 3 F	Two or more <i>Candida</i> -negative samples; no signs of septicemia	
RVVC patients (30)	30.5 (24–44)		Three or more courses of antimicrobial treatment for vaginitis during the preceding year; typical, persistent symptoms (vaginal itching and discharge); one or more <i>Candida</i> -positive vaginal swab specimen cultures	
AEDS patients Group 1 (59) Group 2 (8)	28 (18–61) 29 (19–45)	20 M, 39 F 3 M, 5 F	Diagnosis established by a specialist in dermatology according to the criteria of Hanifin and Rajka (13) and EAACI position statement (14), with eczema typically located in the head, neck, and shoulder region of the skin; positive skin prick test and/or specific IgE antibodies to <i>C. albicans</i> and <i>Malassezia furfur</i> antigens	Systemic antifungal, corticosteroid, or other immunosuppressive treatment
Healthy controls Group 1 (30) Group 2 (6)	71 (32–73) 27 (23–41)	21 M, 9 F 2 M, 4 F	Above-mentioned diseases excluded	

^a The data represent medians (ranges).^b M, male; F, female.

albicans-specific TABM production is elevated in patients with invasive candidiasis and, thus, is associated with severely decreased CMI. Further aims were to evaluate *C. albicans* Cetavlon-mannan and whole extract antigen-specific TABMs for use in the differential diagnosis of *C. albicans*-induced invasive infection and *Candida* colonization and noncolonization and finally, to examine the effect of mannan stimulation on TABM production and IFN- γ and IL-4 mRNA expression by peripheral blood lymphocytes in vitro.

MATERIALS AND METHODS

Study subjects. Thirty-two patients who underwent abdominal surgery at the Department of Surgery of the University Central Hospital of Turku (Turku, Finland) participated in the study. Serum samples were taken when signs of septic or superficial infection were seen during the first postoperative week. The

samples were stored at -70°C and analyzed retrospectively and had no influence on therapeutic decisions. The severity of fungal infection was determined retrospectively on the basis of clinical, microbiological, and autopsy records.

Thirty women from the Department of Gynaecology and Obstetrics of the University Central Hospital of Turku with RVVC were also included the study, and a serum sample was taken after the provision of informed consent.

Fifty-nine patients with atopic eczema dermatitis syndrome (AEDS) from the Department of Dermatology of the University Central Hospital of Turku formed yet another reference patient group for the comparison of serum TABM levels.

Furthermore, in a small group ($n = 8$) of yeast-hypersensitive AEDS patients, the level of TABM production was measured from the cell supernatants of *C. albicans* mannan-stimulated peripheral blood lymphocytes.

Finally, serum samples for TABM level determinations were collected from healthy volunteers ($n = 30$). Samples for peripheral blood lymphocyte stimulations were from laboratory personnel ($n = 6$).

Detailed data on the ages and genders of the study subjects and the inclusion and exclusion criteria used are presented in Table 1.

Antigens. *Candida albicans* (strain CBS 5982, serotype A, obtained from Centraalbureau Voor Schimmelcultuur, Delft, The Netherlands) was cultured on agar plates on synthetic medium as described earlier (45). The mannan antigen (CAM) was purified according to the Cetavlon method (29, 31). The *C. albicans* whole-cell extract, used as an in-house reference (IHR) preparation, was prepared and characterized as described previously (42). *Malassezia furfur* (CBS 7854) was cultured on agar plates on the reduced-fat cream-supplemented medium of Leeming and Notman, as described earlier (22, 23), and the crude cytoplasmic extract was prepared after disruption of the yeast cells in an X-Press chamber (AB Biox, Gothenburg, Sweden), as described by Savolainen et al. (45). All antigens were found to be free of endotoxin contamination by the *Limulus* amoebocyte lysate assay (E-Toxate kit; Sigma-Aldrich, Deisenhofen, Germany).

Monoclonal antibody to human TABMs. The mouse monoclonal antibody (MG3C9-1A12) was prepared and characterized as described previously (24).

TABM determinations by ELISAs. (i) **Detection of serum TABM specific to CAM, IHR, and *M. furfur* crude antigen.** Antigen preparations were diluted in phosphate-buffered saline (PBS; CAM at 0.1 mg/ml, IHR and *M. furfur* at 1.0 mg/ml) and coated overnight at 4°C on separate flat-bottom microtiter plates (Nunc, Roskilde, Denmark). After the plates were washed with PBS-0.05% Tween 20 (PBS-T), they were blocked with 1% human serum albumin in PBS for 90 min at 37°C and rewashed. The serum samples were diluted 1/200 in PBS, 100 µl/well was added in duplicate, and the plates were incubated overnight at 4°C and washed with PBS-T. Mouse monoclonal anti-human TABM antibody was diluted 1/1,000 in PBS-Tween-1% gelatin (PTG; G-2625; Sigma-Aldrich), 100 µl/well was added, and the plates were incubated for 90 min at 37°C and washed. Peroxidase-conjugated rabbit anti-mouse immunoglobulin G (IgG; Dako P-0260; DakoCytomation, Glostrup, Denmark) was also diluted 1/1,000 in PTG, 100 µl/well was added, and the plates were incubated for 90 min at 37°C before they were washed with PBS-T. After the plates were washed with distilled water, 100 µl of tetramethylbenzidine hydrochloride substrate (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD) was added and the reaction was allowed to proceed at room temperature until an optical density (OD) of approximately 1.5 was obtained. The reaction was stopped with 2 M H₂SO₄, and the plates were read at a wavelength of 450 nm with a Multiscan photometer (Labsystems, Helsinki, Finland). TABM OD values were converted to arbitrary enzyme-linked immunosorbent assay (ELISA) units (AU) (0 to 100 AU) by using a linear graph produced with the aid of seven control serum samples with the maximum OD range.

(ii) **Detection of CAM-TABM production in the cell culture supernatants of peripheral blood lymphocytes.** CAM was coated onto ELISA plates as described above. In addition, the blocking was performed as mentioned above. The supernatants of CAM-stimulated and nonstimulated peripheral blood lymphocytes were diluted 1/5 in PBS, 100 µl was added to each well in duplicate, and the plates were incubated overnight at 4°C. The plates were then treated with the antibodies mentioned above, and the reaction was completed as described above.

Mannan ELISA. CAM-specific IgG and IgM antibodies were measured as previously described by Savolainen et al. (44). Briefly, the polystyrene microtiter plates (Nunc) were coated with mannan diluted in PBS and blocked with 1% normal sheep serum in PBS. Next, the plates were incubated for 2 h at 37°C with sera diluted 1/200 in 1% normal sheep serum in PBS; and after repeated washings, they were incubated with alkaline phosphatase-conjugated goat anti-human IgG, anti-human IgA, and anti-human IgM diluted 1/300. After 30 min of incubation with *p*-nitrophenylphosphate in diethanolamine-MgCl₂ buffer, the reaction was stopped with 1 M NaOH and the absorbances were determined at a wavelength of 405 nm with a Multiscan photometer. All tests were performed in duplicate.

IgE determinations. Specific IgE levels against *C. albicans* mannan and whole extract (IHR) were measured by a nitrocellulose radioallergosorbent test, as described earlier (31). *M. furfur* crude antigens were analyzed by a Pharmacia (Uppsala, Sweden) catabolite gene activator protein-radioallergosorbent test, and serum total IgE (S-IgE) was analyzed by a Pharmacia IgE radioimmunoassay.

Lymphocyte stimulations in vitro. Peripheral blood mononuclear cells (PBMCs) were isolated with Ficoll-Hypaque (Amersham Pharmacia Biotech, Uppsala, Sweden), as described previously (21). They were suspended after they were washed in RPMI (Gibco, Life Technologies, Paisley, United Kingdom) supplemented with 10% human type AB serum, 2 mM L-glutamine (Gibco), 100 U/ml penicillin, and 100 µg/ml streptomycin (Nordvacc Media, Skärholmen, Sweden). The cell suspension was diluted in 200 µl of culture medium to 1 × 10⁶ cells/ml and applied to 96-well culture plates (Nunc). A total of 100,000 cells/well were incubated in triplicate at 37°C in a humidified atmosphere with 5% CO₂ for 7 days in the presence of CAM (100 µg/ml) or RPMI.

Cytokine mRNA detection by reverse transcription-PCR (TaqMan assay). The expression of mRNA for IL-4 and IFN-γ in PBMCs, harvested after 7 days of

stimulation with CAM, was determined by real-time PCR (TaqMan assay). The isolation of RNA with Ultraspec (Biotecx Laboratories Inc., Houston, TX) was performed according to the manufacturer's instructions. A First Strand cDNA synthesis kit (Amersham Pharmacia Biotech) was used for the reverse transcription reaction. The amplification of β-actin, IL-4, and IFN-γ cDNA was performed on a MicroAmp optical 96-well reaction plate (Applied Biosystems, Foster City, CA), as described previously (20). The primer and probe sequences were designed by using Primer Express software (Applied Biosystems), and they were the same primers and probes described earlier (20). The PCR was performed in an ABI PRISM 7700 sequence detection system (Applied Biosystems); and TaqMan indices, which indicate the fold increases compared to the level of growth in the unstimulated culture, were thereafter calculated according to the manufacturer's instructions, as presented earlier by Laaksonen et al. (20).

Statistical analysis. Differences in serum TABM levels and the levels of cytokine mRNA expression between study groups were analyzed by the Mann-Whitney U test. The difference in the mean levels of TABM production between colonized and noncolonized patients was calculated by Student's *t* test. Correlations were analyzed by regression analysis.

RESULTS

Statistically significant TABM differences between study groups. The *C. albicans* mannan-specific serum TABM levels of the different study groups are presented in Fig. 1A. The CAM-TABM amount was the highest in the serum of patients with invasive candidiasis, and the difference was significant compared with the amounts in hospitalized patients and healthy controls but insignificant compared with the amounts in *Candida*-colonized patients. *Candida*-colonized patients had a tendency (*P* = 0.091) to have higher CAM-TABM levels than hospitalized patients. The lower level of CAM-TABMs in AEDS patients compared with that in RVVC patients did not quite reach statistical significance (*P* = 0.072). The differences between RVVC or AEDS patients and controls were insignificant.

Comparison of Fig. 1B with Fig. 1A shows that all the *C. albicans* IHR-specific TABM values were lower than the mannan-specific values. Still, the IHR-specific TABM levels in invasive candidiasis patients were significantly higher than those in both hospitalized and healthy subjects. No significant differences were seen between RVVC and AEDS patients and healthy controls.

TABM production in colonized versus noncolonized patients. After the exclusion of confirmed invasive candidiasis cases (*n* = 11) and healthy controls (*n* = 30), the rest of the patients with serum TABM samples (*n* = 110) were also examined for gastrointestinal *C. albicans* colonization based on positive or negative anal swab sample cultures. As further proof of CAM-TABM specificity in colonization and because 54% (59 of 110) of these patients had AEDS, we decided to determine the *M. furfur*-specific TABM levels as well. Colonized patients (*n* = 42) had significantly higher *C. albicans* mannan levels but not IHR- or *M. furfur*-specific TABM levels than noncolonized patients (*n* = 68) (Fig. 2).

Correlation between TABM production and serum immunoglobulins. Patients (*n* = 32) with invasive candidiasis, with *Candida* colonization, and in a postoperative state after abdominal surgery were tested for correlations between their CAM- and IHR-specific TABM and mannan-specific IgE, IgG, and IgM values. Significant correlations were found between both types of TABMs and mannan-specific IgM and IgG. Mannan-specific IgE and TABM levels did not correlate (Table 2).

Serum samples of 46 yeast-hypersensitive AEDS patients

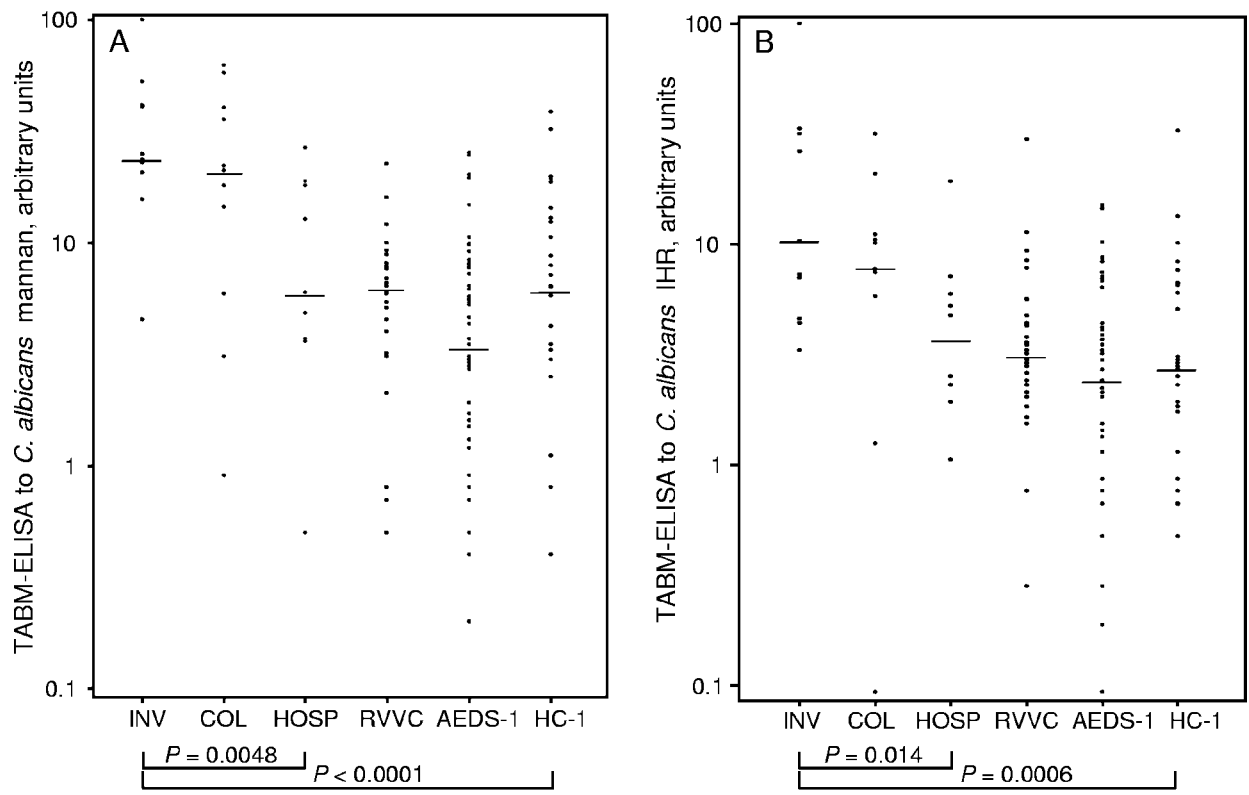


FIG. 1. *C. albicans* mannan-specific (A) and IHR-specific (B) TABM levels of the study groups. INV, invasive candidiasis patients ($n = 11$); COL, *Candida*-colonized patients ($n = 11$); HOSP, hospitalized, noncolonized patients who had undergone gastrointestinal surgery ($n = 10$); RVVC, recurrent vulvovaginal candidiasis patients ($n = 30$); AEDS-1, atopic eczema dermatitis syndrome patients, group 1 ($n = 59$), HC-1, healthy controls, group 1 ($n = 30$). Significant differences ($P < 0.05$, Mann-Whitney U test) are shown. The bars represent medians. TABM levels are expressed as arbitrary ELISA units (see Materials and Methods).

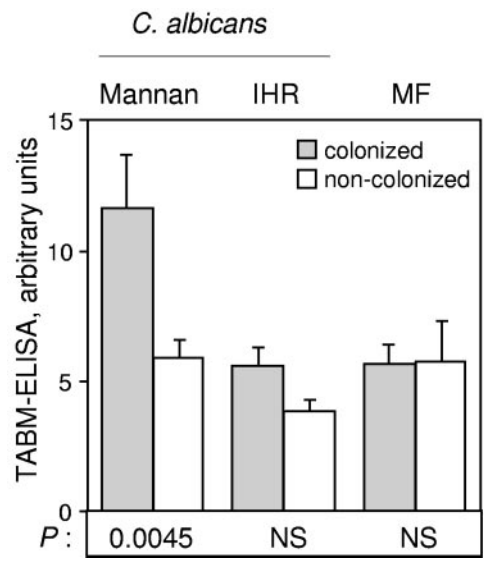


FIG. 2. Levels of antigen-specific TABM to *C. albicans* mannan and whole-cell extract (IHR) and *Malassezia furfur* (MF) determined in *Candida*-colonized ($n = 42$) and noncolonized ($n = 68$) patients. The significant difference of the means ($P < 0.05$, Student's t test) and the standard errors of the means are shown. TABM levels are expressed as arbitrary ELISA units (see Materials and Methods). NS, not significant.

were available for correlation analysis between CAM-, IHR-, or *M. furfur*-specific TABM levels and specific IgE and S-IgE levels. There was not a significant correlation either between antigen-specific TABM and IgE concentrations or between TABM and S-IgE values (data not shown).

All serum samples ($n = 151$) were also studied for antigen-specific TABM level correlations. CAM- and IHR-specific TABM levels had the highest correlation, followed by the correlations between IHR- and *M. furfur*-specific and CAM- and *M. furfur*-specific TABM levels (Fig. 3).

Effect of mannan stimulation on TABM production and cytokine mRNA expression by peripheral blood T cells. The levels of CAM-TABM production from PBMC supernatants and the levels of CAM-specific IL-4 and IFN- γ mRNA expression from the cell pellets of yeast-hypersensitive AEDS pa-

TABLE 2. Correlations between CAM- and IHR-specific TABMs and mannan-specific immunoglobulins

CAM Ig	Correlation for the following TABMs ^a :	
	CAM	IHR
IgM	0.69 (<0.0001)	0.53 (0.0013)
IgG	0.54 (0.0013)	0.41 (0.018)
IgE	-0.0061 (0.97)	-0.0015 (0.99)

^a The values represent the results obtained by regression analysis (P value).

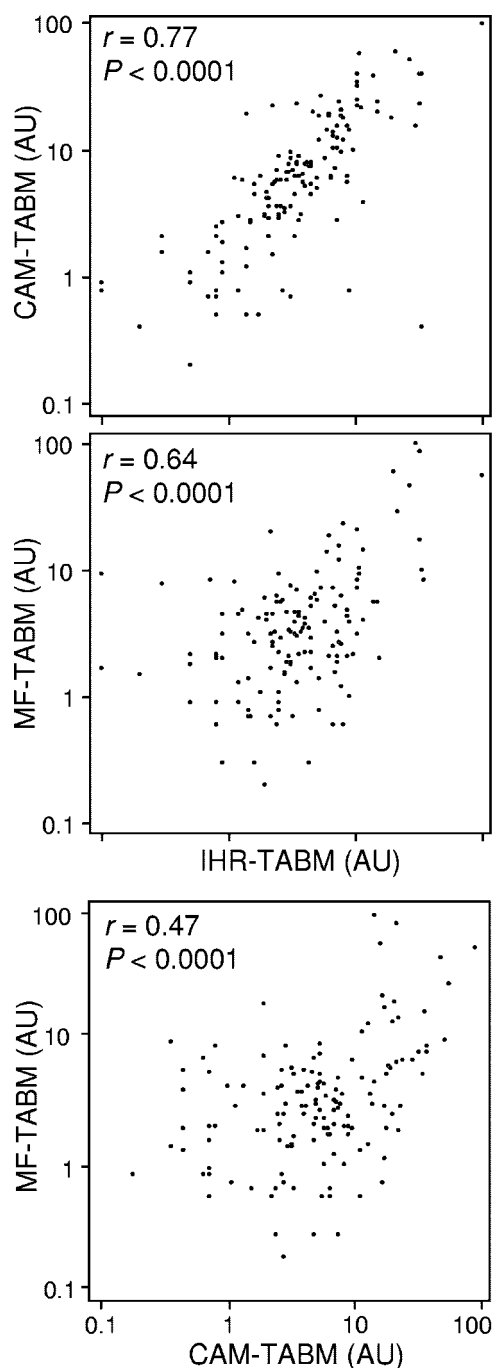


FIG. 3. Correlations (regression analysis) between the levels of antigen-specific TABM to *C. albicans* mannan (CAM) and whole-cell extract (IHR) and *Malassezia furfur* (MF) of serum samples of all study subjects ($n = 151$).

tients ($n = 8$) and healthy controls ($n = 6$) were also measured. It was noticed that mannan stimulation inhibited TABM production and IL-4 mRNA expression and increased IFN- γ mRNA expression in vitro (Fig. 4). When test subjects were divided into high-level ($n = 7$) and low-level ($n = 7$) TABM producers (TABM production indices greater than or equal to and less than -0.78 , respectively), it was seen that IL-4 mRNA

expression was significantly higher in high-level TABM-producing subjects than in low-level TABM-producing subjects (Fig. 5). Instead, there was no difference in IFN- γ mRNA expression between high- and low-level TABM producers ($P = 0.57$).

DISCUSSION

In this study it was demonstrated that *Candida albicans* mannan-specific TABM production was the highest in patients with invasive candidiasis and that CAM-TABM could distinguish *Candida*-colonized from noncolonized patients on a population basis. It was also shown that the CAM-specific and IHR-specific TABM levels correlated with each other and that the CAM-TABM levels correlated with mannan-specific IgM and IgG levels but not with IgE or S-IgE levels. In addition, it was demonstrated that CAM-TABM was directly related to IL-4 mRNA expression but not IFN- γ mRNA expression.

Decreased cell-mediated immunity and the dimorphism of *C. albicans*, i.e., the ability to change between the yeast and the hyphal forms, have been proved to be essential for the development of invasive candidiasis. It was shown in mice that a Th2-skewed immune response with elevated IL-4 and IL-10 production and decreased IL-12 production predisposed patients to invasive *Candida* infection (37–39). The virulence of *C. albicans* has been associated with the formation of hyphae, whereas the yeast form has proved avirulent (27). There is a decrease in the average chain length of mannooligosaccharides, especially of β -1,2-linked mannose residues, in hyphal mannans compared with that in yeast mannans (47). β -1,2-Mannooligosaccharide-induced humoral immune responses (IgM and IgG production) and cell-mediated immune responses (tumor necrosis factor α secretion by macrophages) might protect patients against invasive candidiasis (12, 16, 17). It is likely that DCs or macrophages discriminate between the yeast and the hyphal forms of *C. albicans* and influence the developing cytokine response by the ligation of Toll-like receptors (TLR). This is supported by the observation that TLR4 and TLR2 stimulation promoted Th1-type and Th2-type cytokine production in DCs, respectively (36). Furthermore, intact TLR4 was found to be essential for neutrophil recruitment to the site of *C. albicans* infection, whereas the recognition of *C. albicans* via TLR2 was noticed to induce immunosuppression mediated by increased IL-10 production and the survival of regulatory T (Treg) cells (32, 33). Montagnoli et al. were the first to demonstrate the induction of Treg cells during *C. albicans* infection (28). Based on these findings, it could be suggested that the *C. albicans* yeast form is recognized by DCs via TLR4, leading to a Th1-type immune response and candidal growth restriction, whereas decreased CMI allows hyphal transition, with TLR2-mediated DC activation leading to Treg cell up-regulation and invasive infection. This presumption was confirmed by van der Graaf et al. in a recent publication (52). It seems that invasive candidiasis and *C. albicans*-specific TABM production progress under similar immunological mechanisms, namely, depressed CMI, elevation of Treg cells, and increased Th2-type reactivity (5, 40, 50).

Mannan is a heterogeneous mixture of polymers of various sizes and charges (30). This enables the adequate binding affinity of mannan for plastic, and mannan has been used to coat ELISA plates since the 1980s (10). It was shown in this study

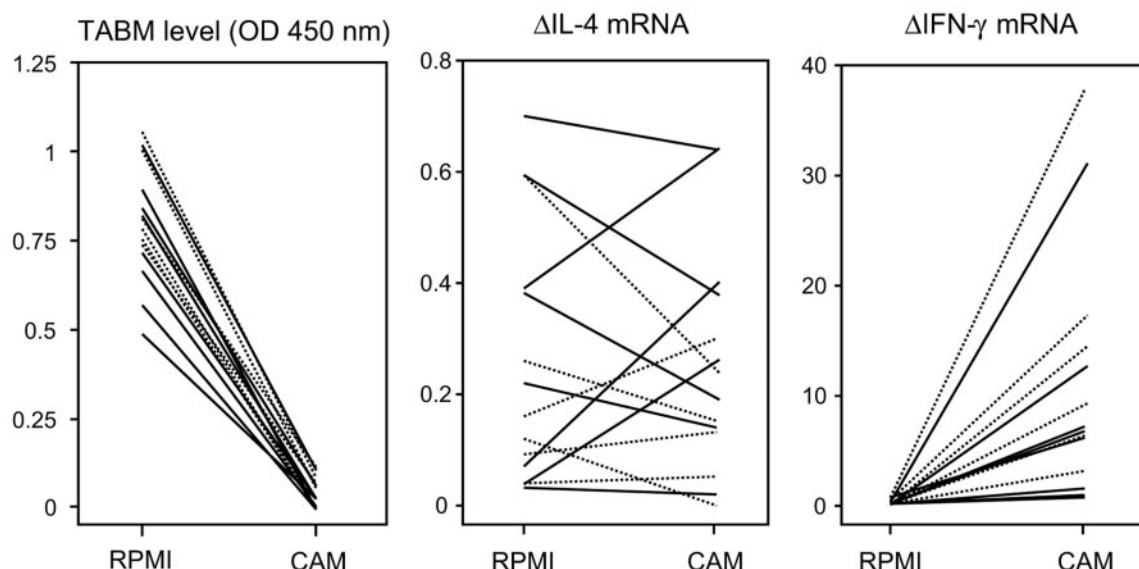


FIG. 4. CAM-TABM levels and the changes (Δ) in IL-4 and IFN- γ mRNA expression in cultures of PBMCs from AEDS patients (solid lines; $n = 8$) and healthy controls (dotted lines; $n = 6$) after 7 days of incubation with (CAM) and without (RPMI) mannan.

that CAM-TABM values were the highest in invasive candidiasis patients, which might indicate a higher Th2-type immune response in their peripheral blood in comparison with that in patients with yeast-hypersensitive AEDS or RVVC. In fact, analysis of cytokines secreted by PBMCs or PBMC-derived T-cell clones of AEDS patients showed a predominance of IFN- γ production over IL-4 production (48, 53). In addition, we previously found that CAM-stimulated PBMCs of AEDS patients produced more IFN- γ and IL-2 than those of healthy controls (43), indicating elevated Th1-type immunity. Unlike in the earlier study by Little et al. (25), we noticed no difference between the CAM-TABM levels of RVVC patients and those of the controls in the present work, which probably

resulted from the use of different patient material. The previous findings of cellular immunity in patients with RVVC have been controversial: after *C. albicans* stimulation, decreased (3, 6), similar (8), or increased (34) levels of lymphoproliferation and IFN- γ production of the PBMCs of RVVC patients compared with those of the PBMCs of controls were suggested.

Positive *C. albicans* anal swab specimen culture results indicating gastrointestinal colonization was noticed in 38% (42 of 110) of the study patients. The culture-positive, colonized patients had significantly elevated serum CAM-TABM values in this study. Since colonization usually precedes bloodstream or invasive *Candida* infection (35), it could be possible that patients with higher CAM-TABM levels are more at risk of invasive candidiasis if they display illnesses with decreased CMI or are being given broad-spectrum antibiotics. Therefore, even though CAM-TABM determination did not distinguish invasive candidiasis from *Candida* colonization, it might provide additional information when at-risk patients are evaluated. The TABM values of *Candida*-colonized patients and noncolonized control patients undergoing gastrointestinal surgery prove that TABM does not act as an acute-phase reactant. In the *Candida*-colonized group, there were no signs of septicemia or other invasive infections, although the TABM level was nearly as high as that in the group with invasive candidiasis. On the other hand, the TABM level in the group of noncolonized control patients who had undergone surgery was not elevated, even though they went through major abdominal surgery, which is known to cause the rapid and long-lasting release of acute-phase reactants, such as C-reactive protein, serum amyloid A, and IL-6 (2).

Mannan polysaccharide, a major yeast allergen, has proved cross-reactive at the level of specific IgE and the skin prick test (19, 41). It was therefore not surprising that the yeast antigen-specific TABM levels correlated as well. What surprised us was that the CAM-TABM level did not correlate with the mannan-specific or total IgE level, whereas it had a good correlation

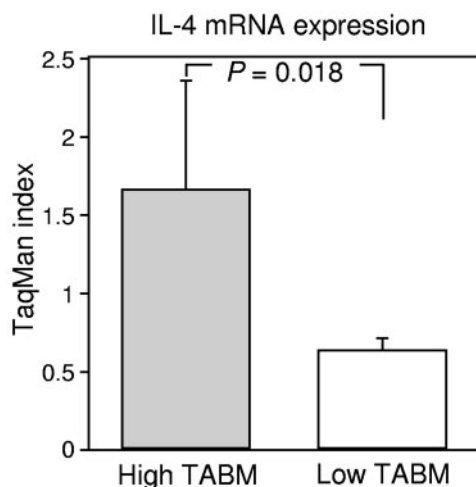


FIG. 5. IL-4 mRNA expression of high-level ($n = 7$) versus low-level ($n = 7$) TABM producers (TABM production indices, greater than or equal to and less than -0.78 , respectively). The index is equal to $\text{CAM}_{\text{TABM}} - \text{RPMI}_{\text{TABM}}$. The P value was obtained by the Mann-Whitney U test. Medians and their absolute deviations are shown.

with the IgG and IgM levels. It is known that the Th2-dependent extrafollicular antibody response initially requires T-cell help but expands in the absence of further T-cell involvement (49). We suggest that once a mannan IgE response is initiated, activated mast cells and basophils could substitute for Th2 cells in the B-cell interaction and further induce mannan-specific IgE production, because they express cell surface CD40L and secrete IL-4, both of which are needed for B-cell maturation (9).

The connection of TABMs to immunoregulatory cytokines (IL-10, TGF- β) and to the Th2-type immune response (5, 26) encouraged us to determine the levels of IL-4 and IFN- γ mRNA expression and TABM production of mannan-stimulated PBMCs. The significant, positive association between mannan-specific IL-4 mRNA but not IFN- γ mRNA and TABM production supports the concept that TABM is a "Th2-type marker." It could be generalized that the increased Th2-type immune response would enable *Candida* colonization, which would proceed into invasive infection if the Th1-type response and, thus, CMI decreased sufficiently. A diminished Th1-type immune response but an equal Th2-type immune response would explain why there was no difference between the CAM-TABM values of *Candida* invasion and colonization. Furthermore, the reduced IFN- γ -induced inhibition of mannan-specific IgE production in patients with invasive candidiasis would allow significant elevation of IgE levels compared with the level of *Candida* colonization previously noticed by Savolainen et al. (44).

In conclusion, this study demonstrated increased *Candida albicans* mannan-specific TABM levels in patients with invasive candidiasis, thus further corroborating the link between T-cell-derived antigen binding immunoproteins and decreased cell-mediated immunity, immunoregulation, and the Th2-type immune response.

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