Infection of Human Oral Epithelia with *Candida* Species Induces Cytokine Expression Correlated to the Degree of Virulence

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A defined and balanced immunomodulatory response is crucial for the protection of mucosal surfaces being in contact with pathogenic microorganisms. This study examined the local host response mechanisms of epithelial cells in experimental *Candida albicans*, *C. tropicalis*, and *C. glabrata* infections by measuring the expression of cytokines at the mRNA and protein level. During the course of infection with active but not with heat-killed *C. albicans* stimulation of the gene expression levels for interleukin-1α, interleukin-1β, tumor necrosis factor, Exodus-2, P-selectin ligand, granulocyte-monocyte colony-stimulating factor, and interleukin-8 was observed by standard and quantitative reverse transcription–polymerase chain reaction. This cytokine pattern may favor a chemotactic and a T helper 1 response. Initial moderate or weak upregulation of these cytokine genes by reverse transcription–polymerase chain reaction was also observed in epithelial infection with the less virulent species *C. tropicalis* and *C. glabrata*. Heat-killed *C. albicans* failed to induce an epithelial immune response. At the protein level, expression of interleukin-8 protein was strongly enhanced during the course of *C. albicans* infection, whereas lower levels were seen with *C. tropicalis* and *C. glabrata*. The different expression patterns of cytokines were associated with differences in virulence of the *Candida* strains. This study's data, therefore, show a correlation between the virulence potential of pathogenic fungi, possibly mediated by specific virulence factors (such as proteinases), and the secretion of epithelial cytokines and chemokines, which may initiate *in vivo* a protective T helper 1 immunologic response and contribute to the recruitment of activated leukocytes and lymphocytes to the site of mucosal infection. ***Key words: C. glabrata/C. tropicalis/IL-8/Sap reconstituted human epithelium/MIP-2.*** ***J Invest Dermatol 118:652–657, 2002***

Mucosal and cutaneous candidiasis are common fungal infections mainly caused by *Candida albicans*. In addition, *C. tropicalis* and *C. glabrata* are emerging pathogens in oral or vaginal infections (Sobel *et al* 2000) The pathogenic behavior of opportunistic microorganisms such as *Candida* species includes the expression of certain virulence factors (Calderone and Fonzi 2001; De Bernardis *et al* 2001; Hube and Naglik 2001). In the last few years molecular approaches have demonstrated the importance of hydrolytic enzymes such as secreted aspartyl proteinases (Sap) for the virulence of *C. albicans*. Experimental infection studies and data from patient samples suggest that especially the isoenzymes Sap1–3 are crucial for local superficial infections (Schaller *et al*, 1998, 1999a,b; De Bernardis *et al*, 1999; Kvaal *et al*, 1999; Naglik *et al*, 1999), whereas Sap4–6 seem to be important in the pathogenicity of invasive candidiasis (Sanglard *et al*, 1997; Borg-von Zepelin *et al*, 1998; Kretschmar *et al*, 1999; Staib *et al*, 2000).

The host defense mechanisms of the innate immune system have been extensively studied in animal models for systemic candidiasis (for review see Romani, 1997), but are poorly understood in mucosal and cutaneous infections. In systemic infection, several animal studies demonstrated that the outcome of the infection depends on the dominant cytokine profile. Cytokine-stimulated T helper (Th)1 cell development leads to resistance and to a protective immunity, whereas a Th2 response is associated with susceptibility (Romani, 2000). More recently, this same trend was also demonstrated in experimental rat vaginitis by De Bernardis *et al* (2000). Furthermore, in a murine model of oral candidiasis an early balanced Th1 and Th2 response was shown to be important for mucosal protection (Elahi *et al*, 2000).

Largely unknown are the immunomodulatory activities during the initial contact between the pathogen (*C. albicans*) and the host (keratinocytes) in local candidiasis (reviewed by Murphy *et al*, 1998). The release of cytokines after contact with the pathogen is believed to be important for the attraction and migration of effector cells (leukocytes) into the epithelial tissue. In previous studies *in vitro* models of oral or cutaneous candidiasis were successfully used based on reconstituted epithelium/epidermis for Sap virulence studies

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(Schaller *et al*, 1998, 1999b, 2000). In this study we examined the local production of cytokines during experimental oral infections with *C. albicans*, *C. tropicalis*, and *C. glabrata*. Using standard and quantitative reverse transcription–polymerase chain reaction (reverse transcription–PCR) and enzyme-linked immunosorbent assay (ELISA) we were able to show that the pattern of cytokine expression correlates with the different virulence phenotypes of these *Candida* species and is also influenced by the activity of *C. albicans*.

MATERIALS AND METHODS

Candida strains, culture media, and conditions In this study the clinical isolates *C. albicans* SC5314 (Gillum *et al*, 1984), *C. tropicalis* DSM4959 (Deutsche Stammsammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany), and a *C. glabrata* strain originally isolated from a female patient with oral candidiasis were used. For the infection of the reconstituted epithelium, inocula (2×10^6) were prepared as previously described (Schaller *et al*, 1998).

Model of oral candidiasis The reconstituted human epithelium for the *in vitro* model of oral candidiasis (Schaller *et al*, 1998, 1999b) was supplied by Skinethic Laboratory (Nice, France). When cultivated at the air–liquid interface in chemically defined medium, the transformed human keratinocytes of the cell line TR146 (Rupniak *et al*, 1985) form an epithelial tissue (mucosa), devoid of stratum corneum, resembling histologically the mucosa of the oral cavity. The mucosa equivalent and all culture media were prepared without antibiotics and anti-mycotics. The epithelial samples were cultured in small inserts on polycarbonate filters. The inserts were transferred into six well plates and the cultures were fed with 1.0 ml maintenance medium under each insert. Epithelial cultures were infected with 2×10^6 *Candida* yeast cells in 50 μl phosphate-buffered saline (PBS). Controls contained 50 μl PBS alone. In the experiments infected and uninfected cultures were incubated at 37°C with 5% CO_2 at 100% humidity for 5, 12, 21, 27, and 36 h.

Experiments with heat killed *C. albicans* To investigate the mechanism of cytokine stimulation the experiments were also performed with heat-inactivated *C. albicans* cells. Killed microorganisms were prepared by heat inactivating at 90°C for 30 min. No live colony was observed on Sabouraud's agar plate after such treatment.

Light microscopy Light microscopical studies were performed as previously described (Schaller *et al*, 1998, 1999b) to evaluate histologic changes during infection. The histologic changes of the mucosa were evaluated on the basis of 50 sections from five different sites for each infected epithelium.

RNA analysis and reverse transcription Reconstituted epithelia were lyzed for 30 s in 1 ml lysis buffer (RNeasy kit, QIAGEN, Chatsworth, CA) with a mechanical blender (Ultraturrax, IKA, Karlsruhe, Germany). During RNA purification genomic DNA was digested with RNase free DNase (QIAGEN). Reverse transcription of total RNA was performed at 1 μg per 10 μl final volume for 55 min at 37°C with 100 U of Moloney murine leukemia virus reverse transcriptase (Life Technologies GmbH, Eggenstein, Germany) in the presence of 1 mM of each deoxynucleotide triphosphate (Pharmacia Biotech, Erlangen, Germany), 10 U recombinant ribonuclease inhibitor (Promega Corp., Madison, WI), 15 mM Tris–Cl (pH 8.4), 60 mM KCl, 3 mM $MgCl_2$, 0.3% Tween 20, 0.1 μg oligo$(dT)_{15}$, and 10 mM β-mercaptoethanol. After a 5 min denaturation step at 95°C the cDNA was diluted to 10 ng per μl with water and stored at –80°C.

Reverse transcription–PCR In a final volume of 20 μl PCR samples contained 5% dimethyl sulfoxide, 60 mM KCl, 10 mM Tris–Cl (pH 8.4), 1.6 mM $MgCl_2$, 0.6% Tween 20, 0.2 mM of each deoxynucleotide triphosphate, 10 pmol of each primer, 0.6 U Taq polymerase and 20 ng of cDNA. Thirty cycles of 1 min at 95°C, 1 min at 65°C, and 1 min at 72°C (5 min final) were performed. The expression of 39 genes in uninfected and infected epithelial cells was measured. For the primer pairs showing no gene expression in this study appropriate controls were used.

Amplifications in 10 μl were visualized by ethidium bromide staining after agarose gel electrophoresis. A 100 bp ladder from Amersham Biotech, Freiburg, Germany was used as the DNA molecular weight marker.

Quantitative reverse transcription–PCR Twenty nanograms of cDNA were analyzed "real time" in a LightCycler (Roche, Grenzach-Wyhlen, Germany) with software version 3.5 using a FastStart DNA Master SYBR Green I kit (Roche) at 3 mM Mg^{2+} final concentration. Annealing temperature and elongation time were optimized for each primer pair. The sequences of the six primer pairs demonstrating a gene expression are given in **Table I**. The corresponding DNA amplificate for each primer pair was serially diluted (6 logs). Aliquots of these dilution series were used to generate standard curves in the same LightCycler PCR run that analyzed the studied cDNA. Quantification for these cDNA was achieved with the LightCycler software.

Both standard and quantitative reverse transcription–PCR were performed in triplicate with similar results.

Table I. The following human primers were used for quantitative reverse transcription–PCR (the sequences are given in the 5′–3′ direction)

IL-1β	5′-primer CGA TCA CTG AAC TGC ACG CTC CG
	3′-primer GGT GAA GTC AGT TAT ATC CTG GCC G
IL-8	5′-GCA GCT CTG TGT GAA GGT GCA G
	3′-GCA TCT GGC AAC CCT ACA ACA G
GM-CSF	5′-GTG GCC TGC AGC ATC TCT GCA C
	3′-CCT GGA CTG GCT CCC AGC AGT C
GAPDH	5′-GCA CCA CCA ACT GCT TAG CAC C
	3′-GTC TGA GTG TGG CAG GGA CTC
IL-1α	5′-CAC TCC ATG AAG GCT GCA TGG
	3′-ACC CAG TAG TCT TGC TTT GTG G
TNF	5′-GGG ACC TCT CTC TAA TCA GCC CTC TGG
	3′-GAC GGC GAT GCG GCT GAT GG

Quantification of interleukin (IL)-8 secretion Epithelial tissues were infected with PBS-washed *C. albicans*, *C. tropicalis*, and *C. glabrata* or treated with PBS only. After 5, 12, 21, and 36 h samples of the maintenance medium surrounding the infected and uninfected epithelial tissues were collected and centrifuged. The amount of IL-8 secreted into the supernatant was determined by an ELISA with optimal concentrations of a mouse anti-human IL-8 monoclonal antibody (G265-5; PharMingen, San Diego, CA) and a biotinylated mouse anti-human IL-8 monoclonal antibody (G265-8; PharMingen) as detecting antibodies. ELISA microtiter plates (Nunc, Lincolnshire, IL) were coated overnight with anti-human IL-8 monoclonal antibodies. After blocking nonspecific binding sites, supernatants were added to the wells and incubated overnight. After several washing steps, biotin-labeled anti-human IL-8 monoclonal antibody was added. Finally, an avidin–biotin–alkaline phosphatase complex (DAKO, Glostrup, Denmark) was added. For signal development the wells were incubated with p-nitrophenylphosphate disodium (Sigma, Munich, Germany), and the optical density was determined at wavelengths of 405 and 490 nm. IL-8 concentrations were calculated from the linear range of standard curves with recombinant human IL-8 (PharMingen).

RESULTS

In preliminary experiments, we examined the expression of the following 39 genes in both uninfected and infected epithelial cells by semiquantitative reverse transcription–PCR: GAPDH, granulocyte colony-stimulating factor, granulocyte-monocyte colony-stimulating factor (GM-CSF), monocyte colony-stimulating factor, hsp-70, the cytokines IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11, IL-13, IL-14, IL-15, IL-16, IL-17, tumor necrosis factor (TNF), Exodus-2, P-selectin ligand, and transforming growth factor (TGF)-β, and the chemokines IL-8, BCA-1, HCC-1, I-309, I-TAC, IP-10, MCP-1, MIG, MIP-1α, MIP-1β, MIP-3β, RANTES, SDF-1β and TARC. We found that of these genes GAPDH, IL-1α, IL-1β, IL-8, TNF, Exodus-2, PSL, GM-CSF, and TGF-β were expressed. We therefore focused the current investigations on these genes.

Basal expression levels of cytokine mRNA Basal expression levels of cytokine mRNA in uninfected reconstituted epithelial cultures were monitored 12 and 36 h after incubation with PBS. Semiquantitative reverse transcription–PCR analysis demonstrated relatively constant mRNA expression levels for GAPDH, IL-1α, IL-1β, TNF, Exodus-2, PSL, and TGF-β genes in all uninfected

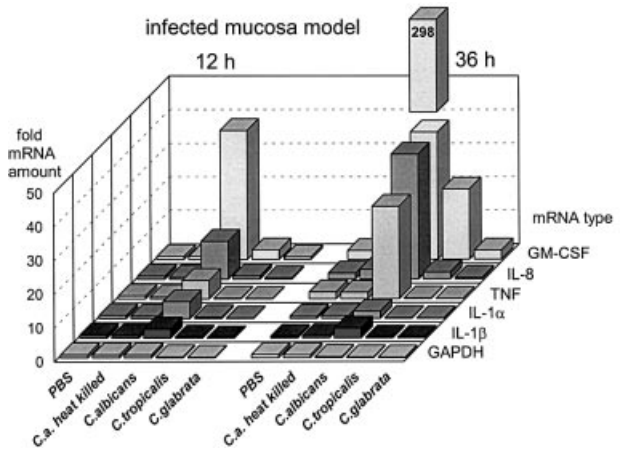

Figure 1. Quantitative analysis of mRNA levels of GAPDH, IL-1α, IL-1β, IL-8, TNF, and GM-CSF in uninfected epithelial samples (PBS) and after infection with active and heat-killed *C. albicans (C.a.)*, *C. tropicalis*, and *C. glabrata*. Relative mRNA levels of infected and uninfected epithelial samples. Expression values of cytokines stimulated by *Candida* were related to the expression level of uninfected epithelia 12 h after incubation with PBS (1.0).

epithelial samples at the indicated time points (not shown). No signals were detected for genes encoding IL-8 and GM-CSF (not shown).

Quantitative reverse transcription–PCR of uninfected samples demonstrated constant levels of mRNA expression for GAPDH, IL-1α, IL-1β, IL-8, TNF, and GM-CSF, and confirmed the results obtained by semiquantitative reverse transcription–PCR for these genes (**Fig 1**).

Expression of cytokine mRNA during epithelial infections with *C. albicans* When expression of cytokine mRNA was investigated during infection of reconstituted epithelial cultures (for 12 and 36 h) with *C. albicans* by semiquantitative reverse transcription–PCR we observed a *de novo* expression of IL-8 and GM-CSF genes and upregulated mRNA levels for IL-1α, IL-1β, TNF, Exodus-2, and PSL genes compared with levels observed on uninfected epithelia. Furthermore, the amount of TGF-β transcripts was decreased, whereas GAPDH mRNA remained constant. *C. albicans* did not stimulate the expression of the remaining genes. Quantitative reverse transcription–PCR using "real-time" PCR demonstrated a significant increase of gene expression for IL-1α, IL-1β, IL-8, TNF, and GM-CSF, 12 h and especially 36 h after infection with *C. albicans* in comparison with the PBS-treated epithelium (**Fig 1**).

Expression of cytokine mRNA during epithelial infections with heat killed *C. albicans* Stimulation with heat-inactivated *C. albicans* failed to induce cytokine upregulation at 12 h and 36 h (**Fig 1**).

Cytokine mRNA expression during epithelial infection with *C. tropicalis* and *C. glabrata* The exposure of epithelial tissue to *C. tropicalis* and *C. glabrata* also altered the expression levels of IL-1α, IL-1β, IL-8, TNF, Exodus-2, PSL, GM-CSF, and TGF-β genes, but the transcript levels and the expression kinetics over the course of infection were different compared with infections with *C. albicans*.

Quantitative reverse transcription–PCR analysis of cytokine expression induced by *C. tropicalis* 12 h after infection demonstrated only a slight increase of mRNA for GM-CSF and IL-8. At 36 h a moderate enhanced expression level was only observed for GM-CSF. Besides a weak increase of GM-CSF 36 h after inoculation *C. glabrata* failed to induce a significant cytokine response (**Fig 1**).

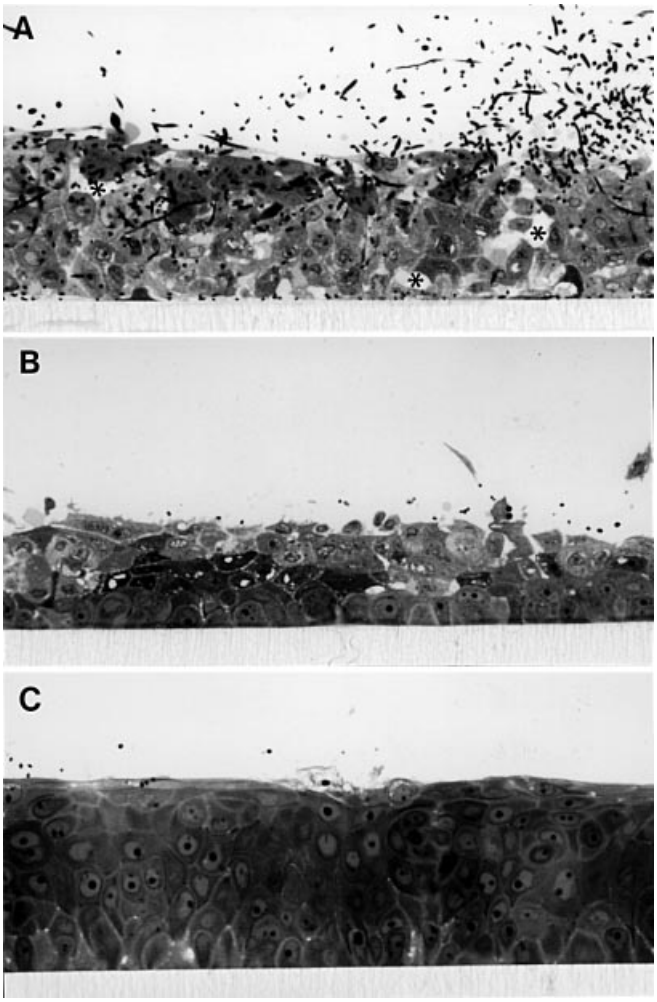

Figure 2. Light micrographs of sections 36 h after infection of reconstituted human epithelium with *C albicans*, *C. tropicalis*, and *C. glabrata*. Invasion of the epithelial cells by *C. albicans* cells (*A*). Multiple vacuoles within the cytoplasm (stars) and enlarged intercellular spaces. Reduced epithelial alterations after infection with *C. tropicalis* (*B*) and *C. glabrata* (*C*). Edema and vacuoles are only seen in the upper cell layers. Invasion and adherence are strongly reduced or blocked (*B, C*).

Morphology of reconstituted epithelium after infection with *C. albicans* Histologic examination of epithelial tissue samples taken 12 and 36 h (**Fig 2*A***) after infection with *C. albicans* demonstrated prominent lesions with edema and vacuolization of the keratinocytes and enlarged intercellular spaces as a sign of spongiosis. In the later stages of infection, *C. albicans* was clearly able to invade all keratinocyte layers of the epithelium. Heat-inactivated *C. albicans* failed to induce any tissue damage (not shown).

Morphology of reconstituted epithelium after infection with *C. tropicalis* and *C. glabrata* Histologic examination of samples 12 and 36 h after epithelial infection with *C. tropicalis* (**Fig 2*B***) and *C. glabrata* (**Fig 2*C***) demonstrated much less dramatic morphologic alterations as compared with infection with active *C. albicans* (**Fig 2*A***). There was mild edema in the uppermost keratinocyte layers with much less spongiosis and vacuolization. Invasion of keratinocytes by fungal cells was completely absent (**Fig 2*B***). Morphologic alterations of the epithelium after infection with *C. glabrata* were not seen or were very weakly positive (**Fig 2*C***).

Stimulation of IL-8 secretion Expression of the IL-8 gene was stimulated by *C. albicans, C. tropicalis*, and *C. glabrata* as compared

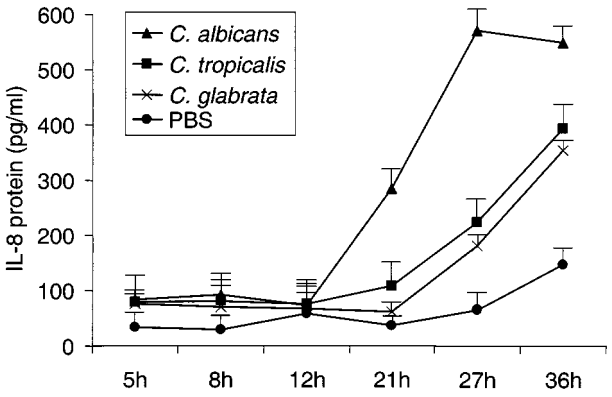

Figure 3. IL-8 protein secretion after stimulation of reconstituted human epithelium with *C. albicans*, *C. tropicalis*, and *C. glabrata*. Mean concentrations ± SD from three experiments are shown.

with uninfected mucosa. The concentrations of the corresponding gene product, IL-8, in the maintenance medium of the epithelial cultures were measured by ELISA after 5, 8, 12, 21, 27, and 36 h incubation time. Values of basal IL-8 expression levels in uninfected epithelium at this time points ranged from 36 pg per ml to 147 pg per ml. Kinetic studies of IL-8 production stimulated by *Candida* cells showed an increase in all three species. Maximal levels were observed for *C. albicans* followed by *C. tropicalis* and *C. glabrata* (**Fig 3**).

DISCUSSION

Cytokine expression stimulated by *C. albicans* has been intensively investigated in models for systemic and vaginal candidiasis by infection of animals (Romani *et al*, 1991, 1992, 1995, 1996; Saavedra *et al*, 1999; Steele *et al*, 1999; De Bernardis *et al* 2000) or in cell culture experiments with endothelial cells (Filler *et al*, 1996; Fratti *et al*, 1996; Orozco *et al* 2000), macrophages (Yamamoto *et al*, 1997), neutrophils (Roilides *et al*, 1995; Romani *et al*, 1995; Romani, 1997; Torosantucci *et al*, 1997; Stevens *et al*, 1998), lymphocytes (La Sala *et al*, 1996), or monocytes (Castro *et al*, 1996; Roilides *et al*, 1996; Chiani *et al*, 2000; Xiong *et al*, 2000; Baltch *et al*, 2001). In short, especially in systemic infections there is evidence that a Th1-type cytokine response correlates with a protective effect. Superficial infections with *C. albicans* are much more common than systemic infections but there are only few studies dealing with the host response in oral and cutaneous candidiasis (Eversole *et al*, 1997; Leigh *et al*, 1998; Ogawa *et al*, 1998; Elahi *et al*, 2000) and especially the initial pattern and functions of cytokine expression in these types of infections are poorly understood (Challacombe, 1994). To characterize the immunomodulatory response of keratinocytes after challenge to *Candida* cells we used a previously established model for oral candidiasis (Schaller *et al*, 1998, 1999b) and compared the expression of cytokines in infected and uninfected reconstituted epithelia. The basal expression pattern of cytokine mRNA observed in uninfected tissue by quantitative reverse transcription–PCR included signals for GAPDH, IL-1α, IL-1β, TNF, Exodus-2, PSL, GM-CSF, IL-8, and TGF-β. Expression of IL-1α, TNF, and TGF-β genes has also been demonstrated in freshly isolated oral or skin keratinocytes (Ansel *et al*, 1990, 1998; Kenney *et al*, 1994; Formanek *et al*, 1998). The similar gene expression pattern found in uninfected reconstituted epithelium indicates that this tissue culture system reflects the *in vivo* situation. In addition, we found basal expression of PSL and Exodus-2 genes, which has not been reported previously.

Exodus-2 selectively stimulates the chemotaxis of T lymphocytes and is preferentially expressed in lymph node tissue and in monocytes (Hromas *et al*, 1997). PSL (P-selectin ligand), which is important for leukocyte recruitment and wound healing, is normally produced by endothelial cells (Subramaniam *et al*, 1997), but expression has also been demonstrated in squamous cell carcinomas (Groves *et al*, 1993). The keratinocytes of the epithelium used in this study are transformed cells derived from a squamous cell carcinoma of the buccal mucosa (Rupniak *et al*, 1985). Therefore one may argue that PSL expression observed in reconstituted epithelium may be due to the transformation of the cells and may not occur in oral epithelia *in vivo*; however, transcripts for the PSL gene have also been found in reconstituted human epidermis (unpublished data) used for a model of cutaneous candidiasis (Schaller *et al*, 2000). In contrast, the keratinocytes used for this epidermal culture system were derived from juvenile foreskins and are not transformed.

The expression of Exodus-2 and PSL in mucosal and epidermal keratinocytes may also play a significant part in host inflammatory response. To investigate this, we performed experimental infection of the epithelium with a highly virulent *C. albicans* and less virulent *C. tropicalis* and *C. glabrata* strains. Several cytokine genes known to be linked with a protective Th1 response, chemotaxis, and activation of macrophages, neutrophils, and lymphocytes *in vivo* were upregulated or downregulated during experimental infection depending on the *Candida* species used. Polymorphonuclear leukocytes play an important part in the host defense against fungal infections and are abundant in superficial candidiasis *in vivo*. Accordingly, keratinocytes challenged with the highly virulent *C. albicans* strain in the *in vitro* model of oral candidiasis showed the strongest increase of mRNA levels for IL-8 and GM-CSF. These chemotactic mediators are involved in the recruitment of neutrophils *in vivo*. Furthermore, the stimulation of a Th1-type cytokine response (TNF) and the downregulation of a Th2-type cytokine (TGF-β) was also shown to be related to the grade of virulence of the *Candida* species. This may reflect the capacity of keratinocytes to detect virulence activities of a potential pathogen and to initiate a protective immune response even in the absence of effector cells such as neutrophils and lymphocytes. The growth factor TGF-β plays an important part in the regulation of cell proliferation, migration, and differentiation (Moses, 1992; Arteaga *et al*, 1996). In a previous study it has been shown that regulation of TGF-β secretion was also associated with the virulence of *C. albicans* strains in a mouse model for systemic candidiasis (Spaccapelo *et al*, 1995). Endogenous production of TGF-β was found to be increased when mice recovered from infection with an attenuated strain but downregulated in lethal infection with a virulent isolate (Spaccapelo *et al*, 1995). A similar downregulating effect of this growth factor by the highly virulent *C. albicans* strain in the *in vitro* models of oral (not shown) and cutaneous candidiasis (unpublished data) indicates the importance of TGF-β for stimulating a protective immune response. There are few studies about chemokine production of keratinocytes stimulated by *C. albicans* (Eversole *et al*, 1997; Ogawa *et al*, 1998). Expression of IL-1α and IL-8 was exclusively detected in samples from patients with oral candidiasis, but not in samples from uninfected individuals (Eversole *et al*, 1997). Furthermore, significantly higher levels of IL-1α, TGF-α, and basic fibroblast growth factor could be demonstrated *in vitro* only after contact of keratinocytes with *C. albicans* but not during interaction with culture medium (Ogawa *et al*, 1998).

The similar results obtained in this study indicate the usefulness of our candidiasis model in mimicking immunomodulatory regulations by host epithelial cells. Differences between the results obtained by semiquantitative and quantitative reverse transcription–PCR might be due the more sensitive analysis of the "real-time" reverse transcription–PCR. The oral model used in this study and an another model of cutaneous candidiasis (unpublished observation) demonstrated corresponding expression of cytokines, which may indicate that similar host defense mechanisms are important for cutaneous and mucosal infections with *C. albicans*. In previous studies both models were used to investigate the contribution of virulence factors to *C. albicans* infections and it was found that proteinase isoenzymes Sap1–3 played a similar role for both,

cutaneous and mucosal infection (Schaller *et al*, 1998, 1999a, b, 2000). As the immunomodulatory activity of the host cells seems to be similar in experimental mucosal and cutaneous *Candida* infections, it is tempting to speculate that similar virulence attributes of the pathogen may have an influence on the defense response of the keratinocytes. Our standard and quantitative reverse transcription–PCR studies demonstrated that enhanced virulence of the *Candida* strains correlated with increased levels of cytokine expression by the host cells. Furthermore, regulation of IL-8 protein secretion was also different in oral candidiasis between the more virulent, proteolytic *C. albicans*, and the less virulent *C. tropicalis* and *C. glabrata* species.

Complete failure to induce epithelial immune response by heat-killed *C. albicans* demonstrated the important role of active yeast cells for adequate cytokine regulation. In contrast, both live and heat-killed bacteria are able to induce enhanced induction of cytokine expression (Paludan, 2000). This suggests that not simply fungal surface molecules, but factors actively produced, released, or modified by living *C. albicans* cells are crucial for the stimulation of cytokine expression.

As the most proteolytic species *C. albicans* caused the most intense immune response, it may be possible that this was due to the secreted proteinases of this fungus.

In previous studies an enhanced immune response was demonstrated for serine proteinases secreted by *Aspergillus fumigatus* that stimulated the expression of IL-6 and IL-8 in airways epithelia cells (Tomee *et al*, 1997; Borger *et al*, 1999; Kauffman *et al*, 2000). One possible host–fungus interaction may be a direct activation of cytokine precursors by fungal proteinases as recently shown for the processing of the IL-1β precursor by *C. albicans* secreted aspartyl proteinases (Beausejour *et al*, 1998). The families of hydrolytic enzymes such as the *Candida* proteinases (Sap1–10) are important virulence factors for the development of systemic, epithelial, and cutaneous candidiasis (Hube and Naglik, 2001). Further investigations are required to study the interaction between these fungal virulence factors and the immune response of the host as they may directly contribute to the development of an inflammatory reaction at the site of infection.

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