Oral Microbiology Immunology 2006: 21: 223–230
Printed in Singapore. All rights reserved

ORAL MICROBIOLOGY
AND IMMUNOLOGY

Expression of interleukin-8 and its receptor IL-8RA in chronic hyperplastic candidosis

A. Ali[1,2], R. Rautemaa[3,4], J. Hietanen[5,6], A. Järvensivu[3], M. Richardson[3], Y. T. Konttinen[7,8,9]

[1]Department of Anatomy/Biomedicum, University of Helsinki, Helsinki, Finland, [2]Department of Pediatric Dentistry, Faculty of Dentistry, University of Garyounis, Benghazi, Libya, [3]Microbiology Unit/HUSLAB, Helsinki University Central Hospital, Helsinki, Finland, [4]Department of Bacteriology and Immunology, Haartman Institute, University of Helsinki, Helsinki, Finland, [5]Department of Oral Pathology, Institute of Dentistry, University of Helsinki, Helsinki, Finland, [6]Oral Pathology Unit/HUSLAB and [7]Department of Medicine/invärtes medicin, Helsinki University Central Hospital, Helsinki, Finland, [8]ORTON Orthopaedic Hospital of the Invalid Foundation, Helsinki, Finland, [9]COXA Hospital for Joint Replacement, Tampere, Finland

Ali A, Rautemaa R, Hietanen J, Järvensivu A, Richardson M, Konttinen YT. Expression of interleukin-8 and its receptor IL-8RA in chronic hyperplastic candidosis. Oral Microbiol Immunol 2006: 21: 223–230. © Blackwell Munksgaard, 2006.

Introduction: Neutrophils are the main opponents of *Candida albicans* in chronic hyperplastic candidosis. They migrate from the circulation to the epithelium where they form microabscesses. We therefore hypothesized that the neutrophil chemokine interleukin-8 (IL-8) might play a role in the neutrophil–*Candida* interaction.
Methods: Biopsies from patients with chronic hyperplastic candidosis ($n = 10$) were stained using the avidin–biotin–peroxidase complex protocol for IL-8 and IL-8 receptor A and were compared to healthy control mucosa ($n = 3$). A set of *C. albicans* agar sections was similarly analysed.
Results: In chronic hyperplastic candidosis lesions IL-8 was strongly expressed in both vascular endothelium and mucosal epithelium. Many resident and immigrant inflammatory cells, including intraepithelial neutrophils, were IL-8 receptor A positive. In addition, IL-8 (or an analogue) was found in the candidal mother cell in chronic hyperplastic candidosis and in agar, whereas the tips of the hyphae expressed IL-8 receptor A (or an analogue).
Conclusion: IL-8 may play a role in the recruitment of neutrophils from the vascular compartment to the epithelial microabscesses. *C. albicans* may have developed an ability to sense IL-8. The IL-8 ligand–receptor interaction may help to direct the growth of the IL-8-receptor-containing tips of the hyphae away from the IL-8-producing candidal cell body (a centrifugal growth pattern to facilitate host tissue penetration). Later, this ability might help to keep the vulnerable hyphal tips away from areas with high concentrations of host IL-8 and candidacidal neutrophils. We suggest that this phenomenon, in contrast to chemotropism, is named chemophobia.

Key words: *Candida albicans*; interleukin-8; neutrophil

Yrjö T. Konttinen, Biomedicum, PO Box 700, FIN-00029 HUS, Finland
Tel.: +358 9 1912 5210;
fax: +358 9 1912 5218;
e-mail: yrjo.konttinen@helsinki.fi
Accepted for publication November 9, 2005

In immunocompromised patients, e.g. those with diabetes mellitus and those undergoing immunosuppressive therapy, fungi can cause a variety of opportunistic infections (11). *Candida* species, in particular, are implicated in nosocomial infections (4, 22). Therefore, fungal infections are sometimes referred to as 'diseases of the diseased' (26). *Candida*, which is a normal fungal inhabitant of the oral cavity, is a major agent responsible for the various manifestations of oral candidosis (28, 30). Infection caused by *Candida* is termed candidiasis or candidosis and represents the most common fungal infection in humans after dermatophytosis (13). *Candida albicans* is considered to be the most common *Candida* species causing infections in humans (20). Recently, the emergence of the life-terminating human immunodeficiency virus infections and the frequent use of immunosuppressive agents have increased the prevalence of candidosis (2). *Candida albicans* can affect any part of the human body but its route of entrance is usually the oral or vaginal mucosa; it also has a role in causing candidaemia (16). Oral candidosis causes intraepithelial inflammation and heavy recruitment of inflammatory cells to the intraepithelial infiltrates, with neutrophils as the main component (10). Oral candidosis has many varieties: it may be atrophic (loss of epithelium causing erythema), pseudomembranous (formation of a slough membrane), or hyperplastic (gain of more layers of epithelium). Chronic hyperplastic candidosis is a form of oral candidosis characterized by hyphal

invasion of the oral epithelium (5, 6); appropriate antifungal therapy should lead to resolution of the condition. Chronic hyperplastic candidosis appears clinically as firm, whitish, palpable lesions, which cannot be rubbed off (27, 32). The neutrophil chemokine interleukin-8 (IL-8) belongs to a group of small (about 8–14 kDa molecular weight), mostly basic substances that have the ability to traffic neutrophils and other phagocytes to the site of candidal infection. We therefore hypothesized that IL-8 and its receptor A form part of the host defence against *C. albicans* and that IL-8 is locally produced by *Candida*-infected tissues, which leads to recruitment of IL-8-receptor-A-positive neutrophils into the hyperplastic epithelial lesions.

Materials and methods

Samples

The local ethical committee approved the study protocol. Ten biopsy samples of oral mucosal lesions were obtained from patients with chronic hyperplastic candidosis who were undergoing examination of their mucosal lesions (Table 1). Histopathology disclosed features typical for chronic hyperplastic candidosis (see Results). Periodic acid Schiff staining of the biopsy samples and/or fungal culture of saliva samples on Dentocult CA® culture medium (Orion Diagnostica, Espoo, Finland) were used to confirm the fungal infection. Three biopsy samples were recruited from healthy individuals to serve as controls (Table 2). Agar sections of *C. albicans* were prepared from stock isolates stored at −20°C and cultivated on commercially available culture plates (Sabouraud Dextrose Agar; Tammer-Tutkan Maljat Oy, Tampere, Finland).

All samples were prepared as consecutive sections, and were semi-quantitatively analysed using a digital light microscope. The main findings (positive cells) were calculated per square area and graded in numerical order.

Immunohistochemistry

Two similar sets of paraffin-embedded sections were used in our experiments comprising both chronic hyperplastic candidosis samples and healthy oral mucosa. Set 1 was stained for IL-8 while Set 2 was stained for IL-8RA. Slides were deparaffinized in xylene, dehydrated through a graded ethanol series and washed in distilled water. Antigen epitopes hidden in the tissue sections by the fixation process were disclosed by immersing the slides in 10 mmol citrate buffer, pH 6.0; then heating them in a microwave at 600 W for 10 min, checking the plastic box after the first 5 min to ascertain that it had enough fluid to evaporate and avoid drying-up of the slides. Slides were left for 30 min at room temperature to cool down. The tissue sections were stained using the avidin–biotin–peroxidase complex protocol. All sections were washed three times in 10 mmol phosphate-buffered saline (150 mmol), pH 7.4, for 5 min per wash. Endogenous peroxidase activity was blocked by immersing the sections in 0.3% hydrogen peroxide in methanol for 30 min. The following steps of the experiment differed for both sets.

For Set 1 the non-specific binding sites were blocked with 1 : 50 diluted normal rabbit serum (Vector Laboratory, Burlingame, CA) for 1 h at room temperature. Then, sections were incubated in 0.2 μg/ml polyclonal goat anti-human IL-8 immunoglobulin G (IgG; IL-8, C-19, sc-1269, Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Control sections were treated with goat IgG antibody of the corresponding isotype against *Aspergillus niger* glucose oxidase (Dakopatts, Glostrup, Denmark), an enzyme which is not present or inducible in mammalian tissues. The sections were left overnight in a humid box at +4°C. The next day, the sections were incubated in biotinylated rabbit anti-goat IgG antibody (dilution 1 : 100, Vector Laboratories), for 1 h at room temperature.

For set 2 the non-specific binding sites were blocked with 1 : 50 diluted normal goat serum (Vector Laboratories) for 1 h at room temperature. Then, sections were incubated in 2 μg/ml rabbit polyclonal anti-human IL-8 receptor A (NLS 806, Novus Biologicals, Inc., Littleton, CO). Control sections were treated with rabbit IgG antibody of the corresponding isotype against *A. niger* glucose oxidase (Dakopatts). The sections were left overnight in a humid box at +4°C. The next day, the sections were incubated with goat anti-rabbit IgG antibody, for 1 h at room temperature.

Both sets were incubated in avidin–biotin–peroxidase complex (dilution 1 : 100; Vector Laboratories) for 1 h at room temperature. Finally, the sites of peroxidase binding were revealed with a combination of 300 μl 3% hydrogen peroxide and 0.023% 3,3′-diaminobenzidine tetrahydrochloride solution (35 mg of DAB in 150 ml phosphate-buffered saline; Sigma Chemical Co., St Louis, MO). All sections were counterstained with Mayer's haematoxylin solution for 30 s, dehydrated in graded ethanol, cleared in xylene and mounted in Diatex (Becker Industrifärg AB, Märsta, Sweden).

To preclude any non-specific binding, we incubated new sections of chronic hyperplastic candidosis in avidin–biotin–peroxidase complex (dilution 1 : 100; Vector Laboratories) for 1 h at room temperature. Then all the steps were followed as mentioned above. No positive staining could be seen in the control sections.

Table 1. Clinical and demographic data of the patients with chronic hyperplastic candidosis

No.	Gender	Age (years)	Location of lesion	Clinical presentation	Additional information
1	M	59	Tongue	Diffuse keratinization	Heavy smoker
2	F	45	Tongue	Red/white lesion	Smarting pain
3	F	59	Tongue	Homogeneous	
4	F	67	Palate	Verrucous	Prosthesis
5	F	55	Cheek	Hyperplastic	Heavy smoker
6	F	53	Tongue	Nodular, indurated	Sharp tooth edges, heavy smoker
7	M	44	Tongue	Ulcerative	
8	M	53	Palate	Verrucous	
9	F	81	Cheek	Papular	Carcinoma of tongue, operated
10	F	85	Tongue	Hyperplastic, verrucous	

M, male; F, female.

Table 2. Clinical and demographic data of the control patients

No.	Gender	Age (years)	Location of the sample	Reason patient presented to clinic
1	F	56	Upper left sulcus mucosa	Resection of left upper incisor (no inflammation)
2 (heavy smoker)	F	57	Upper left sulcus mucosa	Wisdom tooth operation (no inflammation)
3 (smarting pain)	F	44	Lower right sulcus mucosa	Orthodontic treatment (no inflammation)

F, female.

Results

Routine histopathology

Staining of the chronic hyperplastic candidosis sections with haematoxylin and eosin showed parakeratinized epithelium with clear, broad and bulbous rete ridges. In some areas the keratin layer had eroded and the underlying epithelium was exposed. Periodic acid Schiff staining was used to detect the candidal hyphae and together with Dentocult CA® candidal culture was used to confirm the diagnosis. Candidal hyphae were confined to the uppermost layers of the epithelia with varying degrees of penetration. Hyphae were usually growing in a perpendicular direction into the epithelium and were randomly distributed along the epithelial surface, colonizing some areas while absent from others. Individual neutrophils and microabscesses were often seen in the epithelia of chronic hyperplastic candidosis samples.

IL-8 and IL-8 receptor A in host tissues

Staining revealed both IL-8 and IL-8 receptor A in all the chronic hyperplastic candidosis samples and, to a lesser extent, also in the healthy control sections. IL-8 was seen in the microvasculature of the lamina propria in endothelial cells; staining was more intense in the deep than in the superficial vasculature (Table 3, Fig. 1A,C). In contrast to IL-8, the intensity of staining of both deep and superficial endothelial cells for IL-8 receptor A was much weaker and more variable. Some areas were IL-8 receptor A negative, although strongly staining endothelia were also found (Table 4, Fig. 1B,D). Endothelia in the healthy control sections stained for both IL-8 and IL-8 receptor A, although the staining intensity, especially in the deep microvascular endothelium, was usually relatively weak compared to that seen in the chronic hyperplastic candidosis sections (Tables 3 and 4, figures not shown).

In the epithelium, both IL-8 and IL-8 receptor A were found in the basal cell layer, where staining was more intense than in the spinous (middle) cell layer, whereas the parakeratin layer was completely devoid of any immunoreactions except for very sparse staining for IL-8 receptor A in some of the sections (Fig. 2A–D). The epithelium of the healthy controls was generally less intensively stained than the sections from chronic hyperplastic candidosis patients (Tables 3 and 4).

Among inflammatory cells, IL-8 was found in many cell types, including plasma cells, mast cells, macrophages and neutrophils. IL-8 staining of the inflammatory cells was particularly intense in heavily *Candida*-infected and neutrophil-infiltrated samples. Mononuclear inflammatory cells were retained in the submucosa, whereas the polymorphonuclear cells had migrated to the epithelium, where they formed intraepithelial microabscesses containing IL-8-receptor-A-positive neutrophils (Fig. 3A–D). Negatively staining controls confirmed the specificity of the staining (Fig. 1E).

IL-8 and IL-8 receptor A in candidal cells in tissue and in agar sections

In the uppermost layers of the chronic hyperplastic candidosis epithelium *C. albicans*, whenever found, stained positively for both IL-8 and IL-8 receptor A. Candidal cells seemed to be polarized so that the mature blastoconidia (candidal cell bodies) only expressed IL-8 (Table 3, Fig. 4A,C), whereas the hyphal tips were IL-8 receptor A positive (Table 4, Fig. 4B,D).

The simultaneous, and often heavy, staining of the endogenous human IL-8 and human IL-8 receptor A in the epithelium of the chronic hyperplastic candidosis samples made exact topographical evaluation of the staining of the different cell membrane domains of the candidal cells somewhat difficult (because of heavy background staining/noise); for this reason, a set of agar sections bearing cultured *C. albicans* cells were similarly stained. These experiments confirmed that only the mother cells of *C. albicans* expressed IL-8 (Fig. 5A), while IL-8 receptor A was expressed by the outermost parts or the tips of the candidal hyphae (Fig. 5B,C). Neither staining of IL-8 nor of its receptor was seen in the negatively staining controls (Fig. 5D). Staining of the control sections for IL-8 and IL-8 receptor A also revealed positive cells scattered randomly throughout the sections (Fig. 6A,B).

Table 3. IL-8 in chronic hyperplastic candidosis (CHC) and healthy controls

			Epithelium			Lamina propria	
Cases	Candidal mother cell	Candidal hyphae	Keratin layer	Medium layer	Basal layer	Superficial vascular endothelium	Deep vascular endothelium
1 CHC	+++	+	–	++	+++	+	+++
2 CHC	++	++	–	++	+++	++	+++
3 CHC	+++	++	0	++	+++	++	+++
4 CHC	0	0	0	++	+++	++	+++
5 CHC	0	0	–	++	++	+	+++
6 CHC	0	0	–	+	+++	++	+++
7 CHC	++	++	–	++	+++	+	++
8 CHC	+++	++	–	++	+++	+	+++
9 CHC	+++	++	–	+	+++	+	+++
10 CHC	0	0	–	++	++	++	+++
1 Control	0	0	0	+	+	+	+
2 Control	0	0	0	++	++	+++	+++
3 Control	0	0	0	+	+	++	+
Negative control staining	0	0	–	–	–	–	–

0, The structure itself is not present; –, negative; +, some; ++, moderate; +++, high numbers of positive cells.

Discussion

The host reacts to candidal infection (candidosis) by both innate and acquired immunity. The innate host response against candidosis is mediated mainly by neutrophils and macrophages (16). Humoral immunity does not seem to have a profound function in oral candidosis because many studies have failed to show any significant contribution of human anti-*Candida* antibodies (although they do exist) in combating *C. albicans* (8). In mouse models of vaginal candidosis, however, antibodies have been shown to be protective (18, 28). Despite conflicting opinions on the role of cell-mediated immunity, $CD4^+$ T cells are considered to constitute the main defence mechanism of the host against candidosis (6). The role of cytokines, when synthesized, is to augment the defensive role of phagocytes (14). Granulocyte–macrophage colony-stimulating factor and IL-3 stimulate the capability of the monocyte to exert its candidastatic effect (30). In the present study, we were interested in the eventual role of IL-8 and its receptor A in the neutrophil-mediated defence against chronic hyperplastic candidosis. We were able to show that especially the vascular endothelium and oral epithelial cells react to

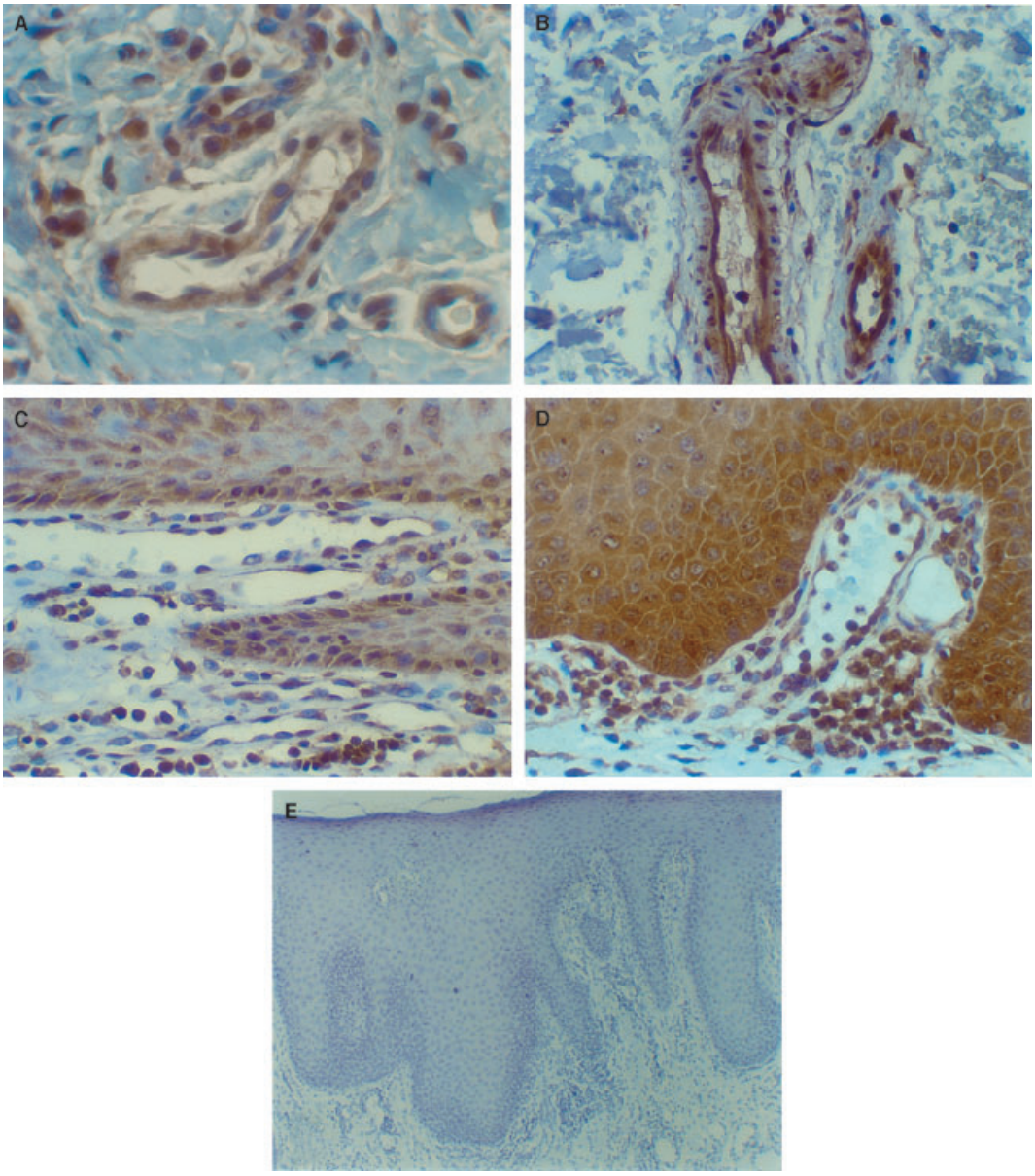

Fig. 1. Staining of connective tissue for IL-8 (A, C), and IL-8 receptor A (B, D) in chronic hyperplastic candidosis oral mucosa. Endothelia lining the neutrophil-rich intravascular compartment can be seen to be strongly positive, in particular in the blood vessel endothelium located deep in the lamina propria (A, B), but they stain less intensely in the more superficial lamina propria (C, D). The negative control does not show any staining (E). Original magnification ×400.

candidosis by secretion of IL-8, which is a chemokine belonging to the CXC subfamily and having potent chemotactic effects on neutrophils both *in vivo* and *in vitro* (3). Although many other cells are involved (14), neutrophils were IL-8 receptor A positive and are often found at sites of *Candida*-infected epithelia (10). They apparently respond to IL-8 by migrating through the vascular wall and lamina propria to the epithelium where the actual candidal hyphae are located. Once they infiltrate the epithelium, the neutrophils form microabscesses and release the antimicrobial contents of their granules extracellularly. One such antimicrobial component is a group of peptides called defensins that, as we have shown in a previous study (1), tend to accumulate in the uppermost layer of the epithelium, forming a rim or shield against the ingress of candidal hyphae. Candidal hyphae, despite their penetrative capacity, seldom reach beyond the spinous cell layer of the epithelium (23). The strong front shield, which is composed of natural antimicrobial peptides, e.g. α- and β-defensins (24) and calprotectin (21), seems to inhibit the growing tips of the hyphae. There has long been a debate, because of conflicting data, as to whether *C. albicans* stimulates epithelial cells to synthesize IL-8 *in vivo* and *in vitro* (12). Whatever the mechanism, in this work we demonstrate that the growth of *C. albicans* in chronic hyperplastic candidosis is associated with relatively strong staining for both IL-8 and IL-8 receptor A. IL-8 is a strong chemotactic stimulus to neutrophils but it may also play a role in candidal cell biology.

To our surprise we discovered that mother cells of *C. albicans* express IL-8

Table 4. IL-8 receptor A staining in chronic hyperplastic candidosis (CHC) and healthy controls

			Epithelium			Lamina propria	
Cases	Candidal mother cell	Candidal hyphae	Keratin layer	Medium layer	Basal layer	Superficial vascular endothelium	Deep vascular endothelium
1 CHC	++	++	±	++	+++	+++	+++
2 CHC	0	0	–	++	++	+	±
3 CHC	++	++	–	++	+++	+++	+++
4 CHC	0	0	–	++	+++	+	±
5 CHC	++	+++	–	+	+++	–	±
6 CHC	++	++	±	+	+++	++	++
7 CHC	+	++	±	+++	+++	++	++
8 CHC	++	++	–	++	+++	±	++
9 CHC	+	++	–	++	++	±	–
10 CHC	0	0	–	+	++	±	+
1 Control	0	0	0	+	+++	–	–
2 Control	0	0	0	++	++	++	++
3 Control	0	0	0	+	++	++	++
Negative control staining	–	–	–	–	–	–	–

0, The structure itself is not present; –, negative; ±, only occasional; +, some; ++, moderate; +++, high numbers of positive cells.

(or IL-8-like protein), while IL-8 receptor A (or IL-8-receptor-A-like protein) was localized mainly in the hyphal tips. We used a basic local alignment search tool (BLAST) to assess whether *C. albicans* has DNA sequences coding these proteins, but no such homologues were found. This, however, does not exclude the possibility that *C. albicans* encodes such proteins because genomic sequencing of *C. albicans* has not yet been completed. Findings first obtained from chronic hyperplastic candidosis tissue sections were confirmed in pure candidal cultures in agar. As we used two different and highly specific antibodies, it seems unlikely that non-specific binding could explain the polarized staining pattern observed. Rigorous staining controls with control IgG antibodies of the corresponding isotype confirmed the specificity of the staining. Finally, the two molecules under study are functionally related, one being the ligand and the other its receptor; this makes it perhaps even more unlikely that this finding is due to chance. Based on our findings we assume that the frequent contact of *C. albicans* with the oral epithelium in both health and disease (because *C. albicans* comprises the major fungal oral microflora in about 25–75% of population) might have driven the fungus to evolve mechanisms that counteract the host's immune defences.

C. albicans has been demonstrated to have a property of contact sensing or thigmotropism (25). This property is

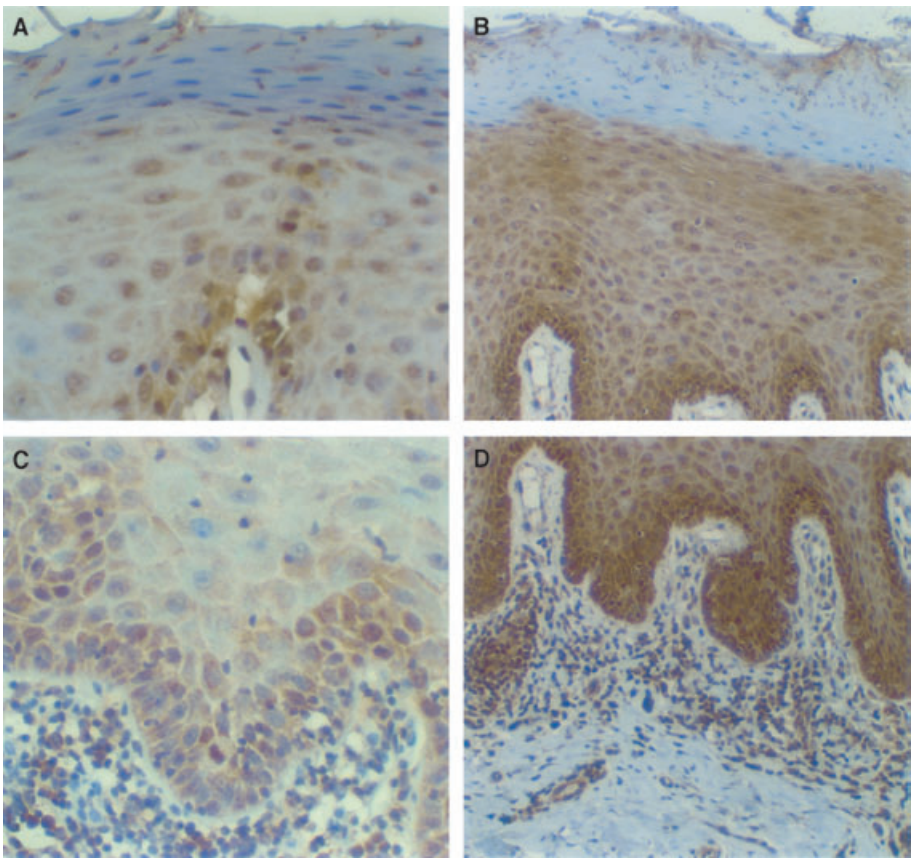

Fig. 2. Staining of oral epithelium and underlying connective tissue for IL-8 (A, C) and IL-8 receptor A (B, D) in chronic hyperplastic candidosis of the oral mucosa. The final destination of the extravasated neutrophils is in the epithelium, where the middle and basal layers are positive (A, B); with the basal layer being particularly intensely stained (C, D). Original magnifications ×400 (A, C), ×200 (B, D).

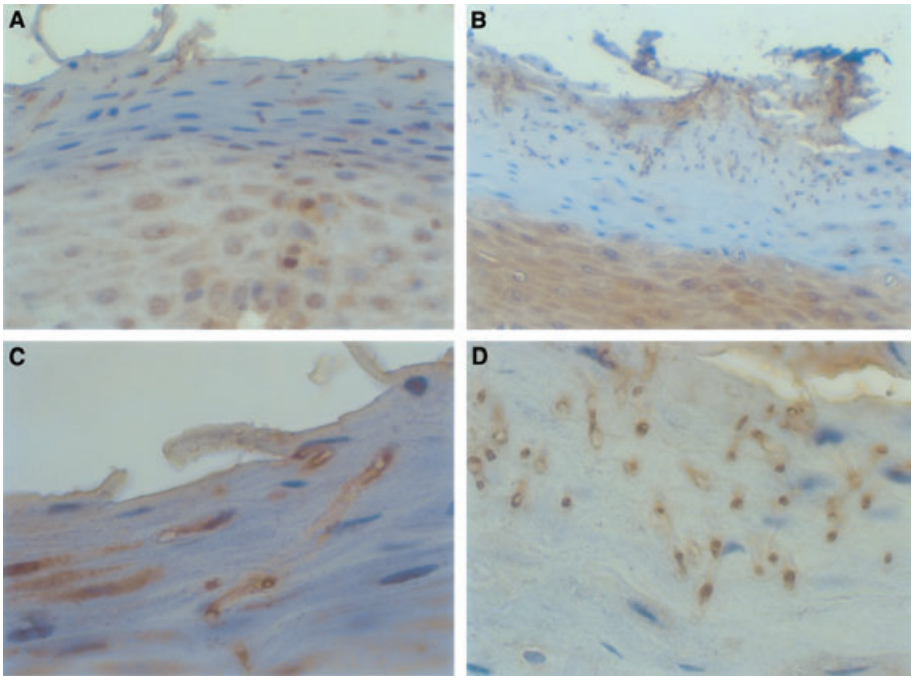

Fig. 3. Staining of *Candida albicans* for IL-8 (A, C) and IL-8 receptor A (B, D) in chronic hyperplastic candidosis of the oral mucosa. Notice the stained candidal hyphae scattered and orientated at different angles in the epithelium. The higher magnification suggests that the candidal mother cells and hyphae express IL-8 (C) and IL-8 receptor A (D), respectively. Original magnifications ×400 (A), ×200 (B), ×600 (C), ×1000 (D).

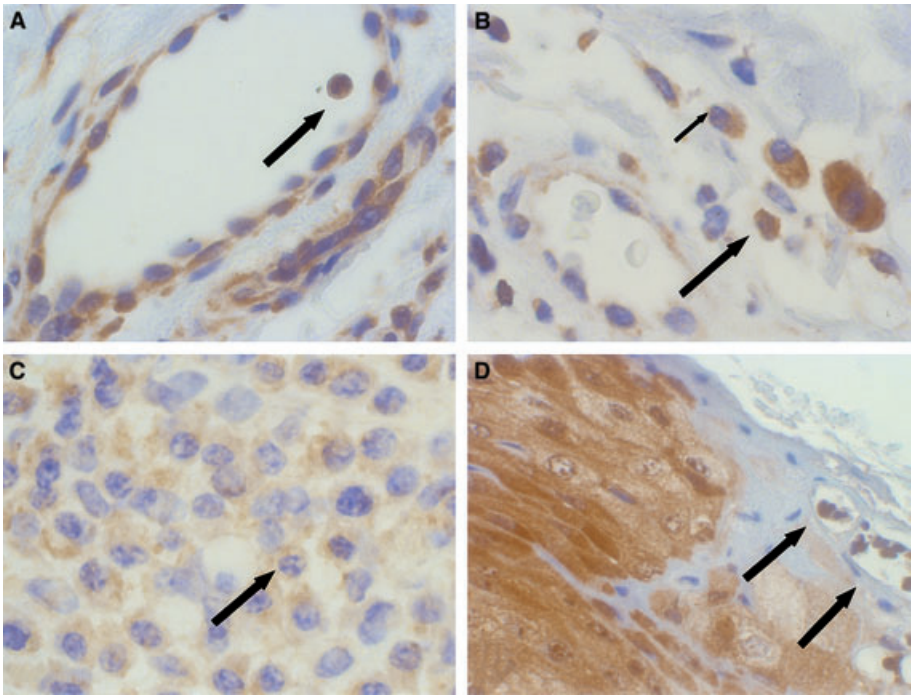

Fig. 4. Staining of inflammatory cells for IL-8 (B, C) and IL-8 receptor A (A, D) in chronic hyperplastic candidosis of the oral mucosa. (A) An IL-8-receptor-A-positive neutrophil (arrow) in the intravascular space, (B) an IL-8-positive neutrophil that seemed to have extravasated perivascularly (arrow); (B) also shows an IL-8-positive perivascular plasma cell (small arrow). (C) IL-8 plasma cells/lymphocytes (arrow) in perivascular infiltrates and (D) IL-8-receptor-A-positive neutrophils after they have migrated to the epithelium, where they form a microabscess (arrow). Original magnifications ×600 (A–C), ×400 (D).

important in the formation of candidal biofilms on intraoral devices such as acrylic dentures (18). A new concept, referred to as chemotropism, has emerged in candidal biology. Davies et al. (8) argued that candidal hyphal tips could elaborate exoenzymes into the underlying host structure and that the hyphal tips, perhaps through plasma membrane recep-

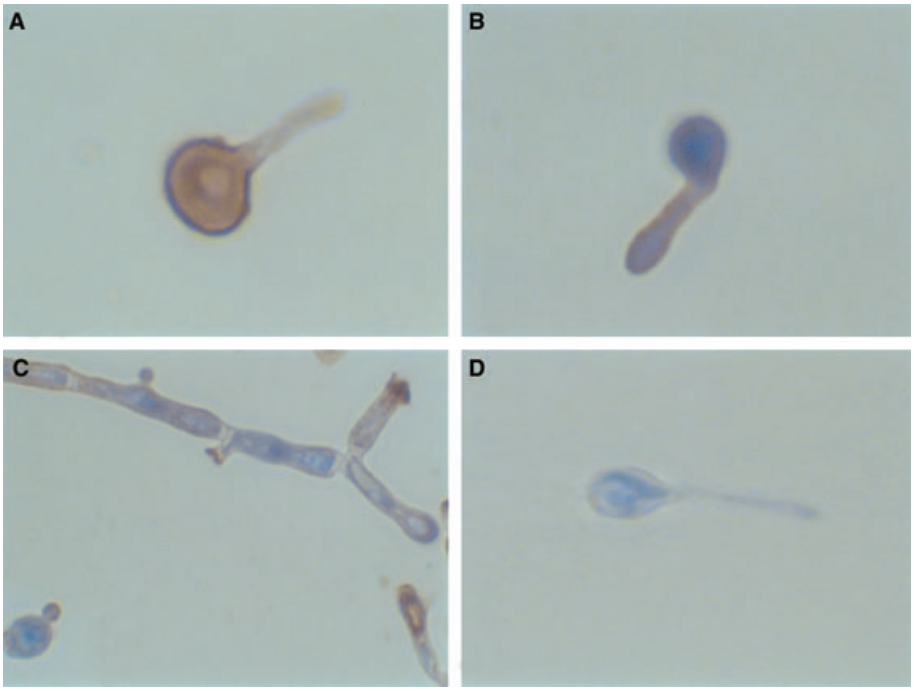

Fig. 5. Staining of *Candida albicans in vitro* for IL-8 (A) demonstrates that the immunoreaction is confined to the mother cell of the fungus, while IL-8 receptor A (B, C) is located mainly at the hyphal tips. The negative control does not show any staining (D). Original magnification ×1000.

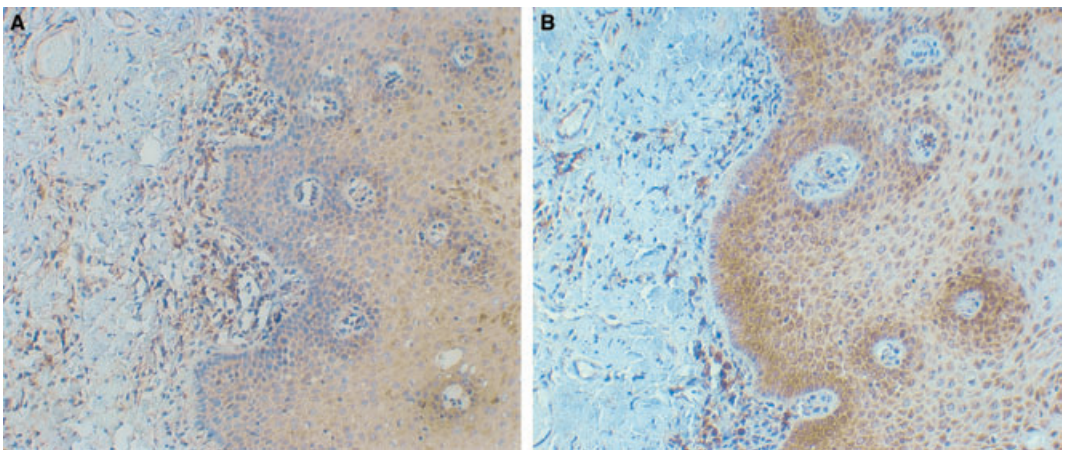

Fig. 6. Staining of a healthy control section with IL-8 (A) and IL-8 receptor A (B). Many immunoreactive cells are seen dispersed in the epithelium and lamina propria, which might indicate the alerted state of the healthy oral tissue to the continuous bathing in a microbiota. Original magnification ×200.

tors, would be able to detect if there are any cellular breakdown products. It might be that *C. albicans* expresses IL-8 receptor A on its hyphal tips so as to be able to sense the eventual presence of IL-8, which might indicate potential danger. This phenomenon could be called chemophobia. It first seemed somewhat paradoxical that the candidal cell body itself produces IL-8 or an analogue. However, if the endogenous candidal IL-8 (or analogue) produced by the mother cell communicates with the IL-8 receptor A (or analogue) at the tip of the hypha, its repulsive effect might help to guide the growth of the hypha in a centrifugal direction, away from the mother cell. Furthermore, as the growing tip of the hypha is very vulnerable compared to the protein- and mannoprotein-enforced wall of the candidal cell, it would seem in the worst-case scenario to be an advantage if a neutrophil-mediated attack is directed against the relatively resistant mother cell/candidal cell wall and is diverted away from the vulnerable tip. When the tip of the hypha has grown to a distance from the mother cell sufficient that communication between the cell body and hyphal tip ceases, this very same ability might help to keep the sensitive hyphal tip away from IL-8-rich areas, which might be or might become heavily infiltrated by neutrophils. Likewise, if the hyphal tip-located IL-8 receptor does not sense any IL-8, *C. albicans* might advance until it is faced by some defence barrier or danger signal. This implies that *C. albicans* might have a strategy to sense the most suitable path for epithelial penetration.

In conclusion, IL-8 and IL-8 receptor A seem to be at work in host defence and

may contribute to the recruitment and migration of neutrophils from the vascular compartment through the lamina propria to the epithelia, where they accumulate and are engaged in anti-candidal defence. At the same time, evolutionary pressure may have driven *C. albicans* to use the IL-8 system in its own survival strategy. IL-8 (or an analogue) was found in the mother cell, whereas IL-8 receptor A (or an analogue) was found at the tip of the hypha. The most straightforward explanation for such an arrangement would be that the candidal cell uses this system first for internal communication to direct the growth of the hyphae away from the cell body, then for external intelligence with the aim of keeping the vulnerable hyphal tip away from danger.

References

1. Ali A, Niisalo S, Hietanen J, Laine M, Rautemaa R, Konttinen Y. Expression of alpha-defensin-1 in chronic hyperplastic candidosis. J Oral Pathol Med 2005: **34**: 347–351.
2. Ashman RB, Papadimitriou JM. Production and function of cytokines in natural and acquired immunity to *Candida albicans* infection. Microbiol Rev 1995: **59**: 646–672.
3. Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines – CXC and CC chemokines. Adv Immunol 1994: **55**: 97–179.
4. Bar W, Hecker H. Diagnosis of systemic *Candida* infections in patients of the intensive care unit. Significance of serum antigens and antibodies. Mycoses 2002: **45**: 22–28.
5. Cawson RA. Chronic oral candidiasis and leukoplakia. Oral Surg Oral Med Oral Pathol 1966: **22**: 582–591.
6. Cawson RA, Lehner T. Chronic hyperplastic candidiasis – candidal leukoplakia. Br J Dermatol 1968: **80**: 9–16.
7. Cenci E, Romani L, Vecchiarelli A, Puccetti P, Bistoni F. T cell subsets and IFN-gamma production in resistance to systemic candidosis in immunized mice. J Immunol 1990: **144**: 4333–4339.
8. Davies JM, Stacey AJ, Gilligan CA. *Candida albicans* hyphal invasion: thigmotropism or chemotropism? FEMS Microbiol Lett 1999: **171**: 245–249.
9. Epstein JB, Truelove EL, Izutzu KT. Oral candidiasis: pathogenesis and host defense. Rev Infect Dis 1984: **6**: 96–106.
10. Eversole LR, Reichart PA, Ficarra G, Schmidt-Westhausen A, Romagnoli P, Pimpinelli N. Oral keratinocyte immune responses in HIV-associated candidiasis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1997: **84**: 372–380.
11. Fridkin SK, Jarvis WR. Epidemiology of nosocomial fungal infections. Clin Microbiol Rev 1996: **9**: 499–511.
12. Hebert CA, Baker JB. Interleukin-8: a review. Cancer Invest 1993: **11**: 743–750.
13. Jarvensivu A, Hietanen J, Rautemaa R, Sorsa T, Richardson M. *Candida* yeasts in chronic periodontitis tissues and subgingival microbial biofilms *in vivo*. Oral Dis 2004: **10**: 106–112.
14. Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. Science 1989: **243**: 1464–1466.
15. Marodi L. Local and systemic host defense mechanisms against *Candida*: immunopathology of candidal infections. Pediatr Infect Dis J 1997: **16**: 795–801.
16. McCullough M, Jaber M, Barrett AW, Bain L, Speight PM, Porter SR. Oral yeast carriage correlates with presence of oral epithelial dysplasia. Oral Oncol 2002: **38**: 391–393.
17. Newman SL, Holly A. *Candida albicans* is phagocytosed, killed, and processed for antigen presentation by human dendritic cells. Infect Immun 2001: **69**: 6813–6822.
18. Nikawa H, Nishimura H, Hamada T, Makihira S, Samaranayake LP. Relationship between thigmotropism and *Candida* biofilm formation *in vitro*. Mycopathologia 1998: **144**: 125–129.
19. Nomanbhoy F, Steele C, Yano J, Fidel PL, Jr. Vaginal and oral epithelial cell anti-*Candida* activity. Infect Immun 2002: **70**: 7081–7088.
20. Odds FC. Pathogenesis of *Candida* infections. J Am Acad Dermatol 1994: **31**: S2–S5.
21. Okutomi T, Tanaka T, Yui S et al. Anti-*Candida* activity of calprotectin in combination with neutrophils or lactoferrin. Microbiol Immunol 1998: **42**: 789–793.
22. Pfaller MA, Jones RN, Doern GV et al. Bloodstream infections due to *Candida* species: SENTRY antimicrobial surveillance program in North America and Latin America, 1997–1998. Antimicrob Agents Chemother 2000: **44**: 747–751.
23. Reichart PA, Philipsen HP, Schmidt-Westhausen A, Samaranayake LP. Pseudomembranous oral candidiasis in HIV infection: ultrastructural findings. J Oral Pathol Med 1995: **24**: 276–281.
24. Sawaki K, Mizukawa N, Yamaai T, Fukunaga J, Sugahara T. Immunohistochemical study on expression of alpha-defensin and beta-defensin-2 in human buccal epithelia with candidiasis. Oral Dis 2002: **8**: 37–41.
25. Sherwood J, Gow NA, Gooday GW, Gregory DW, Marshall D. Contact sensing in *Candida albicans*: a possible aid to epithelial penetration. J Med Vet Mycol 1992: **30**: 461–469.
26. Siqueira JF, Jr, Sen BH. Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004: **97**: 632–641.
27. Sitheeque MA, Samaranayake LP. Chronic hyperplastic candidosis/candidiasis (candidal leukoplakia). Crit Rev Oral Biol Med 2003: **14**: 253–267.
28. Staib P, Michel S, Kohler G, Morschhauser J. A molecular genetic system for the pathogenic yeast *Candida dubliniensis*. Gene 2000: **242**: 393–398.
29. Steele C, Ozenci H, Luo W, Scott M, Fidel PL, Jr. Growth inhibition of *Candida albicans* by vaginal cells from naive mice. Med Mycol 1999: **37**: 251–259.
30. Theiss S, Kohler GA, Kretschmar M, Nichterlein T, Hacker J. New molecular methods to study gene functions in *Candida* infections. Mycoses 2002: **45**: 345–350.
31. Wang M, Friedman H, Djeu JY. Enhancement of human monocyte function against *Candida albicans* by the colony-stimulating factors (CSF): IL-3, granulocyte-macrophage-CSF, and macrophage-CSF. J Immunol 1989: **143**: 671–677.
32. Webb BC, Thomas CJ, Willcox MD, Harty DW, Knox KW. *Candida*-associated denture stomatitis. Aetiology and management: a review. Part 2. Oral diseases caused by *Candida* species. Aust Dent J 1998: **43**: 160–166.