Histopathology, immunology, and serology of oral yeast infections

Diagnosis of oral candidosis

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For diagnostic purposes it is normally not important to obtain a biopsy specimen, since an oral smear from the lesion will yield blastospores and pseudohyphae in abundance. However, in lesions that respond poorly to antimycotic treatment a biopsy should be carried out to detect possible malignant changes in the epithelium. Assessment of cell-mediated immunity against *Candida albicans* and other antigens may be important in patients with severe chronic candidosis to assess the degree of immunocompetence and prognosis. Usually, patients with oral candidosis show only moderately elevated antibody titers in serum and saliva against *C. albicans*, and serologic tests are normally not a diagnostic tool for oral candidosis. However, such tests may be a prognostic instrument in patients with severe oral candidosis who respond poorly to antimycotic therapy. □ *Acute and chronic candidosis; AIDS; angular cheilitis; antibody titers; antigens; biopsy; denture stomatitis; oral smear*

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The process of microbial infection may be regarded as an encounter between the virulence of a microorganism—that is, its ability to overcome host defense mechanisms and to damage host tissues—and the ability of the host to prevent or repel microbial invasion. Histopathologic and immunologic examinations in oral yeast infections are mainly carried out to assess whether the candidal infection is associated with any defect in the immunologic response and to obtain information on the severity of the infection.

Histopathology

Acute candidosis

Histologically, the pseudomembrane in acute pseudomembranous oral candidosis comprises necrotic material, food debris, leukocytes, and bacteria matted and anchored to the oral epithelium by yeasts and pseudohyphae of *Candida albicans*, which are invading the superficial parakeratotic epithelium (a) (Fig. 1). However, *C. albicans* usually does not penetrate deeper than into the stratum corneum. Edema and microabscesses seem to separate the outer layers of the epithelium from each other. The deeper part of the epithelium shows achantosis, and the connective tissue an inflammatory response comprising polymorphonuclear leukocytes, lymphocytes, and macrophages. Similar changes have been observed in animals with experimentally induced oral candidosis (2, 3). For diagnostic purposes it is not important to obtain a biopsy specimen and carry out a histologic examination, since an oral smear from the lesion will show yeasts and pseudohyphae in abundance. It should be recognized, however, that thrush may affect oral malignancies. The histopathology of acute atrophic candidosis is essentially the same as in the pseudomembranous type, but less pseudohyphae are usually observed, whereas the leukocyte infiltration in the connective tissue is heavy.

Chronic candidosis

In patients with chronic oral candidosis of the erythematous, the plaque-like, or the nodular type the parasitic manifestation of *Candida* organisms in the oral mucosa is characterized by formation of hyphae and pseudohyphae that penetrate the epithelial surface (4, 5) (Fig. 2). It still remains an open question whether *Candida* organisms are etiologically related to the lesions or whether they have invaded already established lesions, associated with, for example, heavy tobacco smoking (6). However, the erythematous lesions normally heal after antimycotic treatment, whereas lesions of the plaque type or the nodular type regress considerably.

In HIV-infected patients the acute pseudomembranous candidosis may become chronic and, if untreated, may persist for several months (1). The pseudomembranous lesions may involve any part of the mucosa of the oral cavity.

The fact that a chronic plaque-like or nodular oral candidosis regresses after antimycotic treatment may be regarded as circumstantial evidence that the epithelial changes have been partially induced by yeasts.

It is usually not essential to carry out histologic examinations of tissue sections, since the reliability of the direct smear technique is high in the diagnosis of candidal infection in these lesions. In lesions that respond poorly to antimycotic treatment an oral biopsy should be carried out, as underlying premalignant or malignant changes may be present (Table 1).

Denture stomatitis is an erythematous lesion of the denture-covered mucosa with a multifactorial etiology (7–9). However, *Candida* organisms very often play a significant role as pathogens. Periodic acid-Schiff (PAS)-stained tissue sections of inflamed palatal areas have consistently failed to demonstrate tissue invasion by *Candida* (7). The lesions are characterized by a severe inflammatory reaction, atrophy of the epithelium, and epithelial hyperplasia. Although the epithelial changes may be very pronounced, no cellular atypias have been observed. A palatal biopsy, therefore, is of no importance to confirm a cultural diagnosis or a diagnosis made by examination of a direct smear. On the other hand, *Candida* organisms could be demonstrated in denture sections as constituents of the microbial denture plaque on the fitting denture surface (10). Lesion of denture stomatitis could be reproduced in monkeys when *Candida* organisms were inoculated beneath a close-fitting plate covering the palatal mucosa, but histologic examination of tissue sections showed no evidence of *Candida* organisms within the epithelium (2, 11).

Angular cheilitis is often associated with the presence of a *Candida* infection of the denture-bearing mucosa (12, 13). Furthermore, healing of the lesions has been reported after chemotherapy of the infection of the denture-bearing tissues or when the patients left their dentures out of the mouth (7, 8, 14). It is believed, therefore, that the

Table 1. Laboratory examinations in oral candidosis

	Culture	Smear	Biopsy	Serology	CMI*	Hematology†
Acute pseudomembranous candidosis	+	+	−	−	−	−
Acute atrophic candidosis	+	+	−	−	−	−
Chronic pseudomembranous candidosis	+	+	−	+	+	+
Chronic hyperplastic candidosis	+	+	+	−	−	−
Chronic mucocutaneous candidosis	+	+	+	−	+	−
Denture stomatitis	+	+	−	−	−	(+)
Angular cheilitis	+	+	−	−	−	(+)

+ = Useful; (+) = may be useful; − = inappropriate.
* CMI cell-mediated immunity.
† For example, plasma iron, B vitamins.

infection may start beneath the maxillary denture and from that area spread to the angles of the mouth. However, a decreased resistance of the skin to infection due to maceration, cutaneous disorders, or leukoplakia may also be important predisposing conditions (15).

In patients with clinical signs indicating an acute or chronic oral candidosis the diagnosis is confirmed by semiquantitative cultures or microscopy of direct smears (16) (Table 1). A biopsy and histologic examinations are primarily indicated if malignancies associated with the *Candida* infection are suspected.

Immunology

Differential virulence of species and strains of *Candida* exists. Thus, *C. albicans* is by far the most pathogenic species according to numerous animal experiments (5). *Candida* species have been ranked in accordance with their pathogenicity in experimental animals in descending order as follows: *C. albicans, C. tropicalis, C. stellatoidea, C. parapsilosis, C. pseudotropicalis, C. krusei, C. guilliermondii*, and *Torulopsis glabrata*. Also, strain-related differences of yeast species related to the biotypes have been demonstrated (17). However, even the most virulent *Candida* species, *C. albicans*, occurs ordinarily as a commensal in the human host and is able to invade and damage tissues only when host defenses are locally or systemically impaired. Mechanical irritation from a denture may be such a local factor that could break down the integrity of the mucous membrane; a denture could also prevent salivary anticandidal substances and antibodies from getting into contact with yeast on the denture; finally, a denture could affect the composition of the commensal oral flora in a manner that enhances the propagation of *Candida*. Epithelial changes of the oral mucosa in other sites, due, for example, to tobacco smoking, may also predispose to oral candidosis. However, an altered or depressed immune response is a significant predisposing factor in more severe *Candida* infections, such as in association with acquired immune deficiency syndrome (AIDS), radiation therapy, or treatment with immunosuppressive drugs (5). It may, therefore, be of great importance to assess the patient's level of immune response against *C. albicans* and other antigens, as an impaired immune response could indicate a poor prognosis for controlling the infection. Immunity in superficial candidosis and in oral candidosis is in particular connected to cell-mediated immunity. Thus, in patients with chronic mucocutaneous candidosis a suppressed T-cell function has been demonstrated in most patients—that is, decreased delayed hypersensitivity reaction against *Candida* antigens and a suppression of lymphokine production to stimulation of lymphocytes in vitro by *C. albicans*.

In patients with AIDS, T-cell function is severely impaired and oral candidosis is a very frequent complication; in these patients the infection tends to spread to the esophagus, the latter observation being one of the definite criteria of AIDS (1). The protective significance of an efficient T-cell function has further been illustrated in animal experiments. Thus, in normal animals with experimental palatal candidosis the infection is self-limiting and of 2–3 weeks' duration, associated with an augmentation of the cell-mediated in vitro immune response, and a slight antibody response; no tissue invasion by *Candida* is observed (2, 3). However, in immunosuppressed animals the infection is invasive and persistent and associated with a strong antibody response against *C. albicans*.

Cell-mediated immunity to *C. albicans* antigens has been demonstrated in most human subjects both by the appearance of delayed skin hypersensitivity to *Candida* antigens and by in vitro tests of cellular immunity such as inhibition of leukocyte migration or stimulation of lymphocyte transformation with *Candida* antigens (5). Davenport & Wilton (18) found that the incidence of a delayed hypersensitive skin reaction to a *Candida* extract was lower in a group of 45 patients with *Candida*-associated denture stomatitis than in a group of controls. It was suggested that patients with a negative skin reaction might have acquired the lesions because the protective effect of

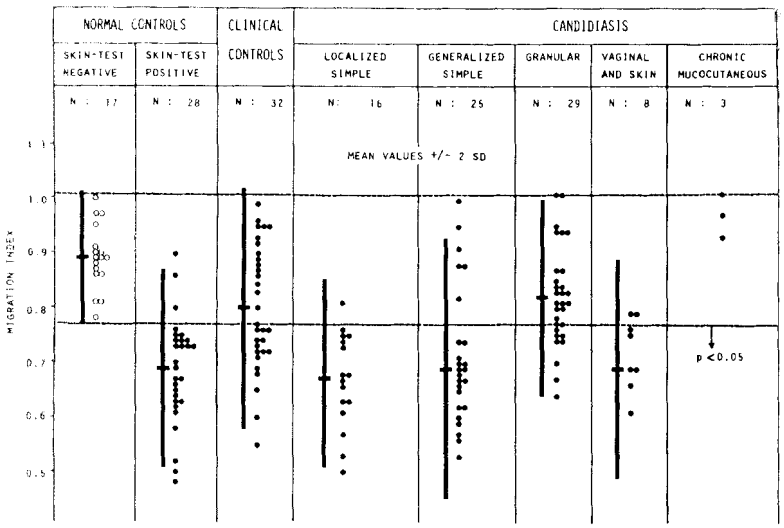

Fig. 3. Cell-mediated immunity against *Candida albicans* assessed by the leukocyte migration test. A considerable number of the patients with candidosis show an impaired cell-mediated immune response against *C. albicans* in vitro. Reproduced, with permission, from Scand J Dent Res 1973;81:372–82.

cell-mediated immunity was absent. In the subsequent study, which used the leukocyte migration test to assess cell-mediated immunity in vitro, Budtz-Jörgensen (19) found an impaired immune response against *C. albicans* quite frequently in patients with *Candida*-associated denture stomatitis (Fig. 3). However, cell-mediated immunity against *C. albicans* was restored after the infection was abolished by treatment with antimycotic drugs (Fig. 4). This reaction indicates that a chronic oral candidosis may lead to a suppression of the cellular immune response in vitro against *C. albicans*. The fact that the suppression was reversible indicates that it is most unlikely that the direct cause of the oral candidosis in these patients was a suppression of cellular immunity against *C. albicans*. In recent studies an intense infiltration by T lymphocytes was demon-

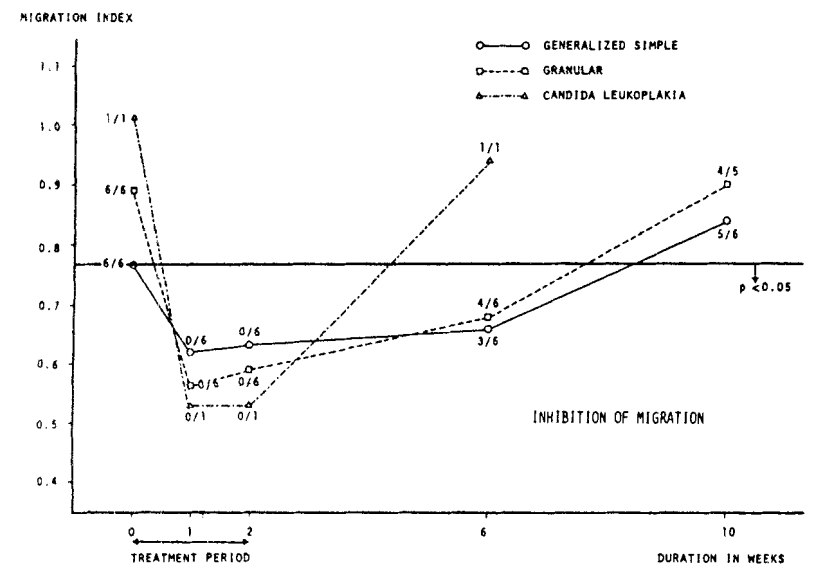

Fig. 4. Cell-mediated immunity against *Candida albicans* assessed by the leukocyte migration test in 12 patients with *Candida*-associated denture stomatitis and one patient with chronic oral candidosis. The fractions give number of infected patients/number of patients. The migration inhibition response became strong when the infection was abolished by topical treatment with amphotericin B. Concurrent with relapse of the infection the migration inhibition reaction was abolished. Reproduced, with permission, from Scand J Dent Res 1973;81:372–82.

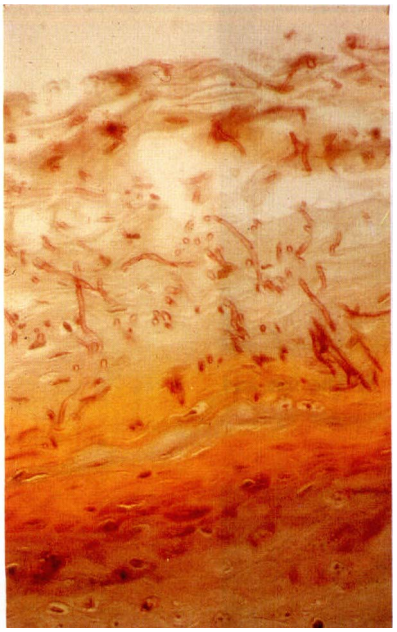

Fig. 1. Acute pseudomembranous candidosis showing plaque of desquamating epithelial cells invaded by pseudohyphae of *Candida albicans*. Reproduced, with permission, from Fiore-Donno G, Section de Médecine Dentaire, Geneva.

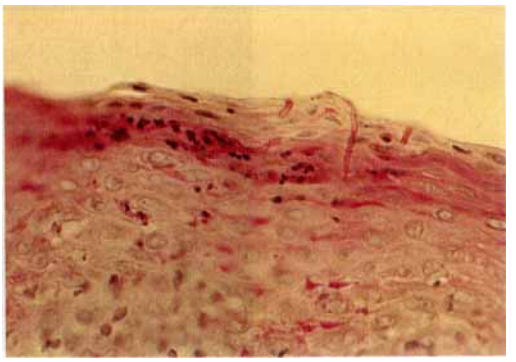

Fig. 2. Chronic erythematous candidosis showing pseudohyphae of *Candida albicans* invading the superficial parakeratotic zone of the epithelium.

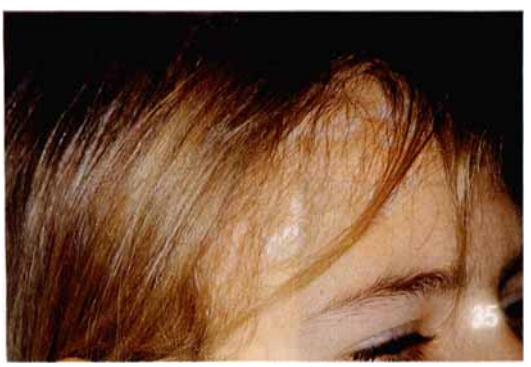

Fig. 5. A 13-year-old girl with chronic mucocutaneous candidosis showing involvement of the scalp. Serum IgG and IgA levels were elevated, the agglutination titer against *Candida albicans* was 1:256, and the leukocyte migration was negative.

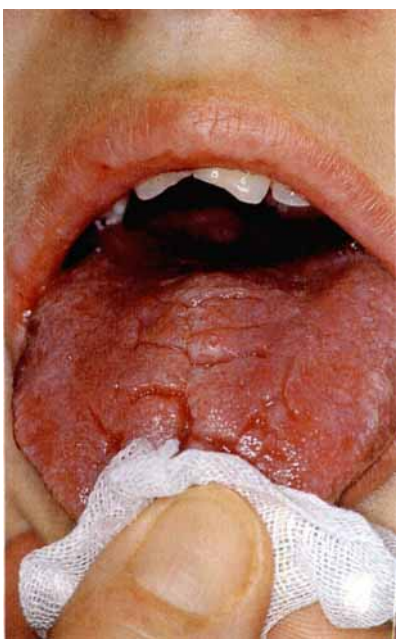

Fig. 6. The same patient as in Fig. 5, showing a severe chronic oral candidosis of the tongue with spontaneous bleeding.

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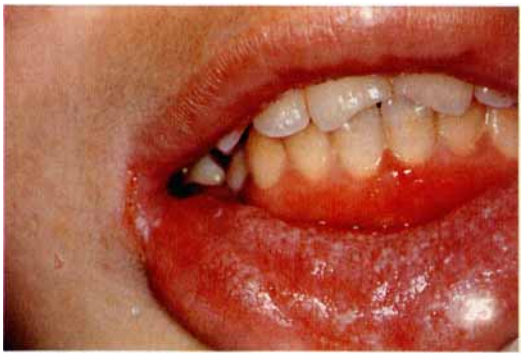


Fig. 7. The same patient as in Fig. 5. The lips and gingiva are also severely infected.

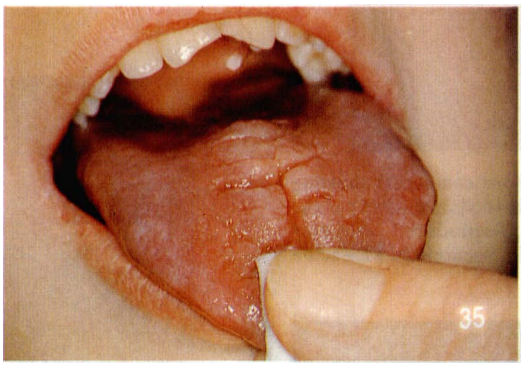


Fig. 8. There is a pronounced amelioration of the lesion of the tongue after topical and systemic treatment with amphotericin B.

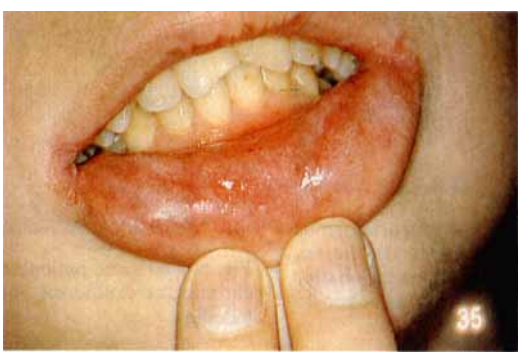


Fig. 9. There is complete clearance of the labial lesions after the antimycotic treatment.

strated in angular cheilitis infected by *C. albicans* and in *Candida*-associated denture stomatitis, indicating an unimpaired cellular immune reaction (14, 20). Immunologic testing of cellular immunity (skin test, lymphokine production) is not an important diagnostic tool in oral candidosis but should be applied if a severely impaired immune reaction is suspected. Reversal of a negative cell-mediated immune reaction to a positive reaction after antimycotic therapy may be a positive prognostic indicator.

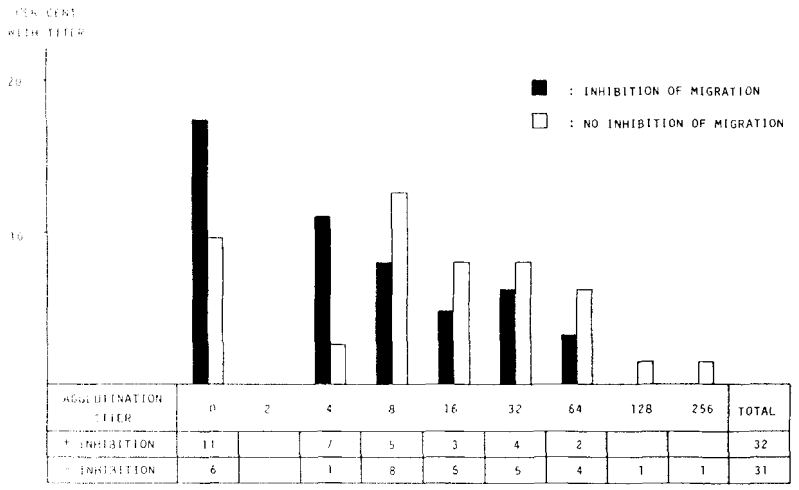
Chronic mucocutaneous candidosis (CMC) is a disorder associated with an impaired cellular immune response and is characterized by a persistent *Candida* infection of mucous membranes, skin, hair, and nails (5). Several types of CMC exist: CMC with familial susceptibility, CMC with endocrinopathy, CMC with both endocrinopathy and familial susceptibility, and CMC with onset later than 10 years of age. The two features generally associated with immunodeficiency in CMC are negative delayed hypersensitive skin reactions against *C. albicans* and decreased lymphokine production by stimulation by *Candida* antigens. However, the patients have normal numbers of rosette-forming T cells, and the T cells are stimulated by phytohemagglutinin and concanavalin A. Thus, these patients have cells that are potentially capable of producing inflammatory mediators to certain antigenic signals but not to *Candida* antigen. Most CMC patients have normal serum and saliva immunoglobulin concentrations and normal or elevated concentrations of *Candida* antibodies (Figs. 5–9). It is important to establish the correct diagnosis from the clinical changes and the immunologic variables, to institute a correct treatment and, if possible, to remove the predisposing factors.

Serology

The detection of *Candida* antibodies in the sera of patients with systemic candidosis has long been considered a rapid and valuable diagnosis of candidosis (21).

The need for a serologic test in addition to conventional mycologic examinations has been motivated by the fact that in 60–85% of cases the deep *Candida* infection was not diagnosed early enough for appropriate therapy to have been instituted (22). In the serologic tests four principal types of *Candida* antigens have been used—namely, whole nonviable yeast cells, *Candida* culture filtrates, cell wall polysaccharides or glycoproteins, and cytoplasmic antigens from mechanically disrupted yeast cells. As immunologic test, the *Candida* agglutinin test, the *Candida* complement fixation test, the *Candida* precipitin and immunofluorescence tests have been used. The usefulness of these tests for diagnosis of deep-seated candidosis has been reviewed by Odds (5). It was concluded that neither qualitative nor quantitative tests are able to distinguish among patients with deep-seated candidosis, oral candidosis, and carriers of *Candida*. It was stressed, however, that in spite of the poor specificity of the serologic tests measurement of changes in antibody concentrations may give valuable information, as the titers may rise during the progression of an infection and fall with its resolution during and after treatment.

In patients with oral candidosis elevated serum anti-*Candida* antibody titers have been observed as compared with controls without affections of the oral mucosa (23). In patients with denture stomatitis a positive relationship has been established between the degree of involvement of the oral mucosa and agglutinating, fluorescent, or the hemagglutinin antibody titers (13, 23, 24). Usually, the raise in the serum anti-*Candida* antibody titer is only moderate in oral candidosis, and similar titers are frequently observed in controls and carriers of *Candida* (Fig. 10). In a study by Axelsen et al. (22), who used crossed immunoelectrophoretic analysis of precipitins to antigens of *C. albicans*, a large number of precipitins was observed in patients with deep-seated candidosis, whereas the number of precipitins was few in patients with oral candidosis and not significantly different from that observed in healthy controls. It is obvious, therefore, that serologic tests for *Candida* are not diagnostic tools for oral candidosis, as the diagnosis could be done more readily by clinical



AGGLUTINATION TITER	0	2	4	8	16	32	64	128	256	TOTAL
+ INHIBITION	11		7	5	3	4	2			32
− INHIBITION	6		1	8	5	5	4	1	1	31

Fig. 10. Relationship between serum agglutination titers and leukocyte migration inhibition against *Candida albicans* in patients with *Candida*-associated denture stomatitis. The negative relationship between the migration inhibition reaction and the serum agglutination titers may reflect that a more severe infection produces high titers and a suppression of the cellular immune reaction of peripheral blood leukocytes against *C. albicans*. Reproduced, with permission, from Scand J Dent Res 1973;81:372–82.

evaluation and by semiquantitative examination of oral smears or cultures. Serial serologic tests might be useful as a guide for the continued therapy and also as a prognostic instrument (24), but in patients receiving immunosuppressive or radiation therapy the antibody response may be suppressed even when the *Candida* infection is severe.

Salivary antibodies

Mucosal membranes are protected by S-IgA, which is the major immunoglobulin in external secretion. S-IgA may play an important role in the defense against oral candidosis, since IgA molecules have been identified on the surface of *C. albicans* and also may inhibit the adherence of *C. albicans* to epithelial cells (25, 26). In patients with oral candidosis increased levels of IgA antibodies to *Candida* have been demonstrated in saliva (23). Thus, there is no evidence that recurrent oral candidosis is associated with an impaired IgA immune response. Pathogenic *Candida* species are able to produce secretory proteinases, which may be important virulence factors (27). Such enzymes have been shown to be able to degrade both serum IgA and S-IgA in vitro (28, 29). In this manner the protective effect of S-IgA is abolished, and the risk that the mucosa is penetrated by microbial antigens is increased.

Conclusions

Semiquantitative cultures and oral smears are the most important tools in the diagnosis of oral candidosis. A microscopic examination of tissue sections is indicated if premalignant or malignant changes of the epithelium associated with the *Candida* infection are suspected. Oral candidosis is frequently associated with a suppression of the T-cell response to *Candida* and other antigens both in vitro and in vivo. A deficient T-cell function may be the direct predisposing condition for the infection. It should be recognized, however, that a reduced T-cell response of peripheral blood lymphocytes against *C. albicans* may be induced by a chronic *Candida* infection, which may be reversible on antifungal therapy and which may not reflect a suppressed T-cell function in the tissues. Serologic tests for *Candida* are not diagnostic tools in oral candidosis since agglutinating, hemagglutinating, precipitating, and immunofluorescent antibodies are found in normal persons without signs of *Candida* infection, and the raise in the antibody response in patients with oral candidosis is only moderate. In selected patients

sequential serologic tests may be useful guides to assess the severity of the infection.

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