Chronic Hyperplastic Candidosis/Candidiasis (Candidal Leukoplakia)

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ABSTRACT: Chronic hyperplastic candidosis/candidiasis (CHC; syn. candidal leukoplakia) is a variant of oral candidosis that typically presents as a white patch on the commissures of the oral mucosa. The major etiologic agent of the disease is the oral fungal pathogen *Candida* predominantly belonging to *Candida albicans*, although other systemic co-factors, such as vitamin deficiency and generalized immune suppression, may play a contributory role. Clinically, the lesions are symptomless and regress after appropriate antifungal therapy and correction of underlying nutritional or other deficiencies. If the lesions are untreated, a minor proportion may demonstrate dysplasia and develop into carcinomas. This review outlines the demographic features, etiopathogenesis, immunological features, histopathology, and the role of *Candida* in the disease process. In the final part of the review, newer molecular biological aspects of the disease are considered together with the management protocols that are currently available, and directions for future research.

Key words. *Candida*, hyperplastic candidosis/candidiasis, review.

(1) Introduction

(1.1) Definition, historical perspectives, and classification

The fact that many oral leukoplakias are associated with *Candida* infections was first reported by Cernea *et al.* (1965) and Jepsen and Winther (1965). However, Lehner (1964, 1967) recognized the presentation of chronic candidal infection in the form of leukoplakia and introduced the term "candidal leukoplakia". The terms "chronic hyperplastic candidosis" (CHC) and "candidal leukoplakia" (CL) appear to have been synonymously used until the mid-1980s (Cawson, 1966a,b; Cawson and Lehner, 1968), but confusion prevailed, since chronic mucocutaneous candidal lesions, encountered in patients with endocrine and immune defects, and affecting the skin and other mucosae, were also described by some as chronic hyperplastic candidosis. Several authors therefore preferred the term "candidal leukoplakia" to describe lesions confined to the mouth alone. In recent times, however, the term "candidal leukoplakia" appears to have lost currency, and most histopathologists prefer the term "chronic hyperplastic candidosis/candidiasis".

To minimize this confusion, Samaranayake (1991) proposed a revised classification where the oral candidosis lesions were subdivided into two main groups: Group I, or primary oral candidoses confined to lesions localized to the oral cavity with no involvement of skin or other mucosae; and Group II or secondary oral candidoses, where the lesions are present in the oral as well as extra-oral sites such as skin (Table 1). The Group I lesions consist of the classic triad—pseudomembranous, erythematous, and hyperplastic variants—and some have suggested further subdivision of the latter into plaque-like and nodular types (Holmstrup and Besserman, 1983).

One distinct clinical difference between the CHC of Group I and that of Group II rests in the fact that the onset of the former is in adulthood, while the latter is almost always first seen in childhood, secondary to relatively uncommon, inherited immune defects (*e.g.*, Di George syndrome). This review pertains to Group I CHC or primary oral candidoses in the above classification. It should be noted, however, that the term "candidal leukoplakia" is still used by some, especially in the medical world. This is not strictly correct, since leukoplakia in an oral context is defined as a white patch that cannot be characterized clinically or pathologically as any other disease (WHO Collaborating Reference Centre for Oral Precancerous Lesions, 1978). Notwithstanding this caveat, we use the terms CHC and candidal leukoplakia (CL) synonymously throughout this review, to facilitate referral to the previous literature that commonly uses the latter terminology.

Interest in CHC appears to have waned globally, as evident from the paucity of recent articles published on the subject. For instance, a search of the Web-based archives of the US National Library of Medicine for publications on "chronic hyperplastic candidosis/candidiasis" during the last decade yielded only 12 publications, of which seven had only indirect reference to the subject. A single paper in Chinese was the only relevant non-English-language publication. A similar search on "candidal leukoplakia" produced only nine papers, six of which made only indirect reference to the topic.

TABLE 1
Classification of Oral Candidosis

Oral Candidosis		
Primary Oral Candidosis (Group I)	Secondary Oral Candidosis (Group II)	
The 'Primary Triad':	**Condition**	**Subgroup**
Pseudomembranous (mainly acute)	Familial chronic mucocutaneous candidosis	1
Erythematous (acute/chronic)	Diffuse chronic mucocutaneous candidosis	2
Hyperplastic (mainly chronic)	Candidosis endocrinopathy syndrome	3
- Plaque-like	Familial mucocutaneous candidosis	4
- Nodular/speckled	Severe combined immunodeficiency	5a
***Candida*-associated lesions**	Di George syndrome	5b
Denture stomatitis	Chronic granulomatous disease	5c
Angular cheilitis	Acquired immunodeficiency syndrome	6
Median rhomboid glossitis		
Linear gingival erythema		

Modified from Samaranayake (1991).

(1.2) Epidemiological and demographic aspects of CHC: incidence, age, gender, and site variations

The most common and arguably the classic clinical presentation of CHC is a white plaque that cannot be rubbed off and presenting most frequently in the commissural regions of the oral mucosa (Fig. 1). However, other oral sites can be infrequently affected. The lesion can be differentiated from oral leukoplakia of idiopathic origin, since appropriate antifungal therapy usually leads to resolution of the condition.

The epidemiological data on CL are linked to those of oral leukoplakia in general. At this point, it is appropriate to review the definition of oral leukoplakia. After considerable confusion in the preceding decades, leukoplakia was defined in 1978 under the auspices of the World Health Organization as a white patch or plaque that cannot be (rubbed off or) characterized clinically or pathologically as any other disease (World Health Organization, 1997).

The prevalence of oral leukoplakia as a whole in unselected population samples has been reported to range from 0.2-4.9% in rural India (Mehta *et al.*, 1969, 1972), 3.6% in rural Hungary (Bruszt, 1962), and 11.6% in Sweden (Axéll, 1976). According to several reports, the leukoplakias that harbor candidal hyphae appear to constitute from 7 to 50% of all leukoplakias (Jepsen and Winther, 1965; Roed-Petersen *et al.*, 1970; Daftary *et al.*, 1972; Roed-Petersen and Pindborg, 1973; Bánóczy, 1982; Krogh *et al.*, 1987b). Lehner (1971) has suggested that differential counts of pyroninophilic monocytes be carried out to distinguish between candidal and non-candidal leukoplakias. The clinical and histological criteria laid down by Cawson and Lehner (1968) in their original description of CL appear to be fulfilled by only 10% of leukoplakias encountered in the mouth (Burkhardt and Seifert, 1977; Arendorf, 1984).

It has already been mentioned that CHC of Group I candidosis is a disease of adulthood. The age range of a cohort studied by Arendorf *et al.* (1983) in Cardiff was from 31 to 81 years, and the majority of the patients were more than 50 years old.

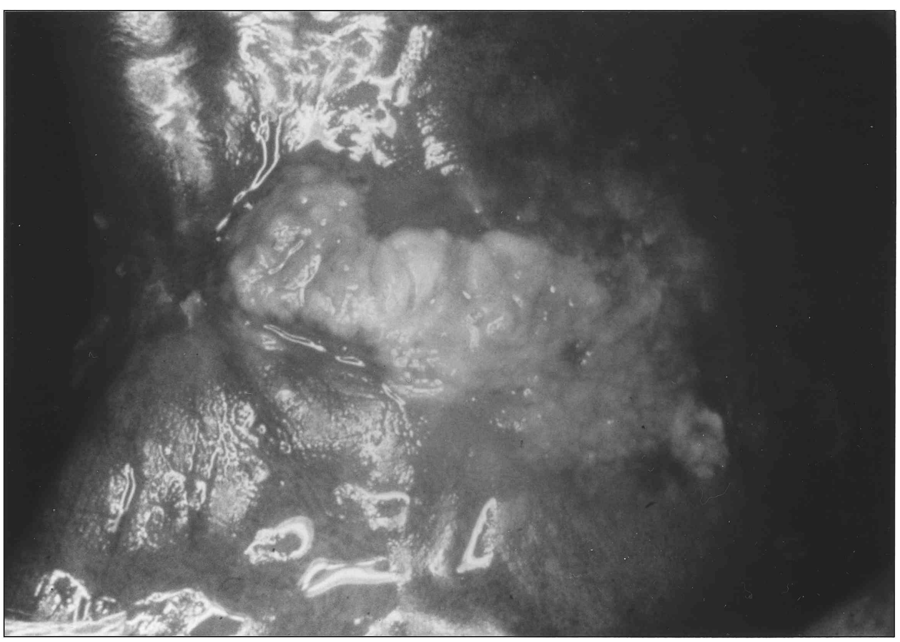

Figure 1. The classic presentation of chronic hyperplastic candidosis at the commissures of the oral mucosa.

Gender distribution among oral leukoplakias varies, with M:F ratios ranging from 4:1 to 85:1 in different parts of India (Mehta *et al.*, 1969) and 20:1 in Sweden (Axéll, 1976). Not surprisingly, this preponderance of the male gender among affected individuals is reflected in the case of CL as well. Cawson and Lehner (1968) showed a M:F ratio of 2:1 among those affected by CL. Walker and Arendorf (1990) pointed out that the latter ratio was in conformity with the gender ratio found among UK tobacco smokers in a study by Arendorf *et al.* (1983).

CL affects the following oral sites in decreasing order of frequency: the buccal commissures, cheeks, palate, and the tongue (Williams, 1969).

(1.3) Pathogenic attributes of *Candida*

Regardless of the type of candidosis, the ability of *Candida* species to persist on mucosal surfaces of healthy individuals is an important factor contributing to its virulence. This is particularly important in the mouth, where the organism has to resist the mechanical washing action of a relatively constant flow of saliva toward the esophagus. It is of course true that infection by an opportunistic pathogen such as *Candida* is dependent not only on virulence factors of the organism but also equally, or indeed more, on host factors. Such host factors, both local and systemic, will be discussed below. However, a brief review of the virulence factors of *Candida* species in general is relevant at this juncture.

The adherence of *Candida* to oral mucosal surfaces is critical to its persistence in the mouth and thus can be a prelude to the study of its virulence (Kennedy, 1988). The ability of the organism to adhere to mucosal and artificial surfaces in the mouth has been extensively studied (for a recent review, see Samaranayake and Ellepola, 2000). The factors that contribute to the adhesion of *Candida* fall into three major groups, as shown in Table 2.

TABLE 2

Factors Affecting Candidal Adhesion to Host Tissues or Prostheses

Factors related to yeast cells
Medium/cultivation
Phenotype
Germ tubes/hyphae
Extracellular polymeric material (EP)
Floccular/fibrillar surface layers
Mannan
Chitin
Hydrophobicity
Cellular lipids
ALS (agglutinin-like substances; up to 9 are known)
Factors related to host cells
Cell source
Mucosal cell size and viability
Fibronectin
Fibrin
Sex hormones
Yeast carriers *vs.* patients with overt candidosis
Environmental factors affecting adhesion
Cations
pH
Sugars (especially galactose)
Saliva
Humoral antibody and serum
Low concentrations of antifungal agents
Bacteria
Lectins

Modified from Samaranayake and Ellepola (2002).

Seen from a general perspective, candidal adherence appears to be a complex process, with no single factor independently contributing to adherence. Several surface components on the yeast cell wall appear to interact with oral epithelial cells initially through a variety of physical forces such as van der Waals' interactions, hydrophobicity, and electrostatic bonding. Subsequent intimate host-cell interactions are mediated through lectin-like proteins of the yeast cell wall that interact with a terminal sugar of the glycoproteins of the human epithelial cell, which acts as a receptor for the former (Critchley and Douglas, 1987a). A fibrillar surface component of the yeast cell wall, a strain-specific mannoprotein named adhesin, has been identified by Critchley and Douglas (1987b) and subsequently demonstrated electron microscopically (Marrie and Costerton, 1981; McCourtie and Douglas, 1981). Recent studies have also uncovered a gene family termed ALS (Agglutinin-like substances) that plays a critical role in candidal adhesion (Hoyer, 2001).

From the standpoint of CL where hyphal forms of *Candida* are seen invading the superficial layers in histopathological specimens, it is of interest that the adherence of the hyphal phase of *C. albicans* is greater than that of blastospore-phase cells (Samaranayake and MacFarlane, 1982; Tronchin *et al.*, 1988). Kimura and Pearsall (1978) and Tronchin *et al.* (1988) have suggested that structural or biochemical changes in surface components such as adhesins may account for this phenomenon. However, Kennedy and Sandin (1988), on the basis of their study of candidal adhesion in a variety of media and growth conditions, have proposed that fungal adhesins and hydrophobicity play a negligible role in candidal adhesion to epithelia.

Most strains of *C. albicans* and *C. glabrata* display a phenomenon known as 'switching', which enables the organism to change phenotype and display various colonial morphology and physiological properties (Di Menna, 1952; Slutsky *et al.*, 1985; Soll *et al.*, 1987; Soll, 2002). The biological significance of this phenomenon is poorly understood. There is some evidence, however, to imply that switching systems may contribute to the pathogenicity of *Candida* by enabling the organism to survive in adverse host environments and alter its surface antigenicity, thus evading the host immune mechanisms and even escaping antifungal treatment. It has also been proposed that switching may selectively augment candidal adhesion and increase the capacity for tissue penetration and secretion of proteinases and phospholipases (for a recent review, see Soll, 2002).

Another major virulent attribute of *Candida* is its ability to invade superficial layers of the epithelium, aided, in particular, by their hyphal appendages. Early workers have shown that the candidal proteinases (secreted aspartyl proteinases [SAP]) and lipases are particularly concentrated at the tips of these hyphal elements (for a recent review, see Ghannoum, 2000). Since adherence of *C. albicans* to epithelial cells is optimal at acidic pH (Samaranayake and MacFarlane, 1982), and such an environment is conducive for SAP production, acidic conditions prevalent intra-orally, especially in diseased states, appear to promote candidal virulence (Samaranayake and MacFarlane, 1985). Extracellular proteolytic activity of *C. albicans* was first discovered by Staib (1965), who first pointed out the role of SAPs in the pathogenesis of candidosis (Staib, 1969). Borg and Ruechel (1988) found a strong association between fungal adherence to the buccal mucosa and the activity of the SAPs. Borg *et al.* (1984) found that proteolytic candidal cells were highly cytotoxic for human monocyte-like cells, whereas non-proteolytic blastospores caused little toxicity. Several studies have shown that many protective salivary proteins such as lactoferrin, lactoperoxidase, and immunoglobulins, especially IgA1 and IgA2, are susceptible to degradation by SAPs of *Candida*. The same is true for hemoglobin (Ruechel, 1986, 1990). Since *Candida* SAPs have keratinase-like activity *in vitro* (Negi *et al.*, 1984), it is likely that this property would enable the organism to invade orthokeratinized mucosa. Zimmermann (1986) has demonstrated the ability of the fungus to invade even the blood vessels in the submucosal tissues.

As has been mentioned, candidal SAPs require a low pH (*circa* 5.5) to act optimally. Ruechel (1990) points out that suitable acidic conditions may exist in sites of poor blood supply, while others have shown that *Candida* can secrete organic acids into its micro-environment, thus creating a favorable milieu for proteolytic activity (Samaranayake *et al.*, 1983). The same authors have shown that such acidification is particularly prominent in budding yeasts and in the tips of growing filaments, a feature which is significant in the context of CL. Secretory lipases of *Candida* species were identified by Werner (1966), who described it among strains of *C. albicans* (*C. stellatoidea,* now *C. albicans*) and *C. tropicalis.* Samaranayake *et al.* (1984b,c) and Kantarcioglu and Yucel (2002) have shown that the phospholipase activity differed significantly among isolates of *C. albicans*. Previous workers have shown that the phospholipase activity was highest where the hyphae were in direct contact with the cell membranes at the invading front of the lesion (Pugh and Cawson, 1977). In conclusion, it is tempting to speculate that the tandem activity of both phospholipases and SAPs of *Candida* is likely to play a key role in host invasion by this ubiquitous fungus. Unfortunately, however, there is a scarcity of data on this front and also on the qualitative differences of SAPs and phospholipases among different *Candida* species.

(2) Host Factors in Candidal Leukoplakia/Chronic Hyperplastic Candidosis

It is well-known that *Candida* species are normal commensals in up to 50% of the healthy population (Samaranayake and MacFarlane, 1990). It is therefore conceivable that many local and systemic host factors may operate *in vivo* to facilitate the conversion of this harmless commensal to a pathogenic organism. These are adequately dealt with elsewhere in relation to the many clinical variants of oral candidosis (Samaranayake, 1990). In the following section, we attempt to clarify the role of these factors in the pathogenesis of CHC.

(2.1) LOCAL PREDISPOSING FACTORS

It is likely that a major element leading to the initiation of CHC is a breach of the integrity of the host oral mucosa. Oral mucosa is an intrinsically thick, impervious integument with a multiplicity of defense mechanisms, including the relatively recently discovered antibiotic peptides in epithelial cells, termed "defensins" (Zasloff, 1992). However, these defenses are not uncommonly breached by a variety of factors. Trauma to the mucosa from natural as well as artificial teeth is relatively common. A denture, especially a maxillary appliance, poses an additional threat, since it acts as a potent reservoir of *Candida* in comparison with the prosthesis-free oral cavity (Budtz-Jørgensen, 1990). Walker and Arendorf (1990) have proposed a schematic model for the pathogenesis of candidal leukoplakia wherein occlusal friction acts as a co-factor leading to the initial oral keratosis. Since it is a common observation that most commissural leukoplakias, predominantly characterized as CHC, occur along the occlusal line, it is tempting to speculate that occlusal trauma or friction is a prime protagonist of CHC. However, we did not find any direct evidence in the literature to suggest that occlusal friction or trauma from normal or artificial dentition is significantly high in cases of CHC. There is only a single report (Arendorf *et al.*, 1983) that provides some indirect support for this hypothesis, where a significantly higher proportion of candidal leukoplakia patients had dentures and wore them continuously day and night. Interestingly, though, in angular cheilitis, which often accompanies CHC of the adjacent commissural mucosa, maceration at the commissural angle of the mouth has been recognized as a contributory factor leading to the condition (Scully and Cawson, 2001).

Epithelial changes of the oral mucosa, such as atrophy, hyperplasia, and dysplasia, may compromise the mucosal barrier and may facilitate candidal invasion, especially in the event of epithelial atrophy (Samaranayake, 1990). However, there are no studies that have compared the thickness of epithelium in relation to candidal invasion. On the contrary, there is a considerable volume of evidence that candidal invasion itself may provoke a hyperplastic response (*vide infra*).

Reduced salivary flow rate in diseased states such as Sjögren's syndrome, or during cytotoxic therapy and radiation therapy, has been shown to favor increased oral carriage of *Candida* and a concomitant increase in candidal infection (MacFarlane and Mason, 1973; Martin *et al.*, 1981; Samaranayake *et al.*, 1984a, 1988). Although no definite link between xerostomia and CHC has been reported, it is conceivable that CHC may be precipitated with other variants of candidosis in xerostomic patients, provided that conducive factors co-exist.

Apart from the quantitative changes in salivary flow discussed above, qualitative changes of saliva, such as its glucose content and pH, are also factors that may influence oral candidal colonization and probably indirectly affect the genesis of CHC (Samaranayake, 1990). High glucose content of saliva and its low pH have been shown by several workers to favor oral candidal colonization (Knight and Fletcher, 1971; Shipman, 1979; Arendorf and Walker, 1980; Samaranayake *et al.*, 1984b, 1986).

(2.2) TOBACCO SMOKING AND CHEWING HABITS: EPIDEMIOLOGICAL EVIDENCE OF RELATIONSHIP TO TOBACCO USAGE

Bánóczy *et al.* (2001) have provided strong evidence for the role of smoking in the development of both oral cancer and oral leukoplakia in Hungarian cohorts. Cross-sectional studies show a higher prevalence of leukoplakia among smokers, with a direct dose-response relationship between tobacco use and oral leukoplakia in general.

In one study, Arendorf *et al.* (1983) have reported that all of their 53 candidal leukoplakia patients were smokers with a very high degree of statistically significant prevalence ($P < 0.001$) than the non-smoking controls. They also pointed out that areas protected from the direct insult of the tobacco smoke, such as the denture-bearing mucosa of the palate, were relatively unaffected in general.

In a study involving Indian villagers, it has been reported that 98% (48 out of 49) of those with *Candida*-infected leukoplakias smoked or chewed tobacco (Daftary *et al.*, 1972). Previously, Pindborg *et al.* (1967) found a strong correlation between bidi smoking and commissural leukoplakia in Indians. Pindborg (1980) asserted, citing evidence from Hoffmann *et al.* (1974), that bidi smoke has a higher content of noxious agents than does cigarette smoke.

There is contradictory evidence, too, with regard to the relationship between tobacco smoking and oral candidosis in general. Several researchers claim to have found no correlation between smoking habit and oral candidosis (Gergely and Uri, 1966; Coleman *et al.*, 1976; Bastiaan and Reade, 1982; Oliver and Shillitoe, 1984). Indeed, Beasley (1969) has reported three isolated cases where the onset of oral thrush coincided with the cessation of the smoking habits of the patients.

Although it might appear that evidence for a direct relationship between oral candidosis as a whole and smoking is far from irrefutable, the studies cited above show conclusively that smoking habit has a direct link at least to commissural leukoplakia and, by inference, to CHC. Furthermore, Arendorf and Walker (1980) have shown that *C. albicans* was isolated more frequently from the mouths of smokers than from non-smokers. So why does tobacco smoking favor oral candidal colonization? Analysis of the available data indicates that this could be due to: (a) induction of increased epithelial keratinization (Zimmermann and Zimmermann, 1965; Mosadomi *et al.*, 1978; Bánóczy, 1982); (b) reduction in salivary immunoglobulin A levels (Bennet and Reade, 1982); and (c) possible depression of polymorphonuclear leukocyte function (Kenney *et al.*, 1977).

(2.3) Systemic Predisposing Factors (Diabetes, Immunological Defects, Nutritional Factors)

(2.3.1) Diabetes mellitus and candidal leukoplakia

Only a minority of patients with candidal leukoplakia have associated medical conditions, including diabetes mellitus (Walker and Arendorf, 1990). Furthermore, an analysis by the present authors of a large number of reports on oral candidal infections among diabetics reveals that candidal leukoplakia is fairly uncommon in this patient group (unpublished data). Nevertheless, an appraisal of the general relationship between oral candidosis and diabetes mellitus cannot be considered irrelevant in the context of this review.

Experiments on animal models for oral candidosis have shown that diabetes increases the susceptibility to candidal infestation (Andriole and Hasenclever, 1962: Hurley, 1966; van Cutsem and Thienpont, 1971; for a recent review, see Samaranayake and Samaranayake, 2001).

Willis *et al.* (1999) have recently provided comprehensive data on the subject of oral candidosis in diabetics. Their study, involving 414 insulin-dependent diabetes mellitus patients, has revealed that 77% of diabetics carried *Candida* species in their oral cavity, with *C. albicans* being the species most frequently isolated, and that 40% patients colonized with candidal species had no clinical signs of oral candidosis. They further showed that where oral candidosis was present, erythematous candidosis was the most common clinical presentation, and candidal load was not associated with age, sex, or glycemic control. On the contrary, they found candidal load to be significantly increased in smokers but not in denture-wearers or those with clinical signs of oral candidosis. However, there had been some reports contradictory to some of these findings. Arendorf and Walker (1979) and Tapper-Jones *et al.* (1981) have previously shown that the candidal carriage rate in diabetics who wear dentures is higher than that in dentate diabetics. Farman, in 1976, observed that poorly controlled diabetic individuals have a higher oral carriage of *Candida* than do better-controlled patients. But Tapper-Jones *et al.* (1981) and Fisher *et al.* (1987) were unable to show any correlation between poor glycemic control and oral candidal carriage.

Several reasons for the high oral candidal carriage in diabetics have been suggested. First, the high salivary glucose levels in diabetics may favor the growth of yeasts (Knight and Fletcher, 1971). However, the studies cited above which demonstrated a lack of correlation between glycemic control and oral candidal carriage fail to substantiate this. Another possible mechanism that has been proposed is that the adhesion of *Candida* to buccal epithelial cells of diabetic patients is significantly greater than that of cells obtained from non-diabetic controls, implying that there are intrinsic qualitative changes in the cell-surface receptors of diabetics that modulate yeast adhesion (Darwazeh *et al.*, 1990).

Kumar *et al.* (1980) have shown that germ tube formation of *C. albicans* is higher in sera of diabetics. Yet, the same authors did not observe a similar finding with regard to saliva of diabetic patients (Kumar *et al.*, 1982).

Finally, Wilson and Reeves (1986) have shown that candidacidal activity of neutrophils may be defective, particularly in the presence of glucose—a further mechanism that may aid candidal infestation in diabetics.

(2.3.2) Immunological aspects of chronic hyperplastic candidosis

Researchers in oral candidosis and clinicians interested in the condition have always been intrigued by the existence of the several clinical variants of the entity, despite the single etiologic agent causing the disease. Reichart *et al.* (2000) have attempted to address this issue in the light of currently available information on the histopathological and immunocytochemical aspects of the disease, as well as the pathogenic characteristics of the fungus. They surmise that known and still-unknown differences in the virulent attributes of the fungus, such as the production of extracellular proteinases, within and between species may play a contributory role in the genesis of the clinical variants. They also suggest that hyperplastic candidosis could be considered a superficial cellular reaction to the pathogen, which cannot entirely be eradicated by the systemic or local host immune response. Similar hypotheses have been proposed by others with regard to cutaneous candidosis (Sohnle and Kirkpatrick, 1978).

At this juncture, it is appropriate to review the immune mechanisms that operate in candidal infections in general with particular relevance to chronic hyperplastic candidosis.

As with any other infection, both specific as well as non-specific immune mechanisms are involved in the defenses against human candidal infections. The role of some of the non-specific and local host factors has already been alluded to. These are summarized in Table 3.

As far as specific immunity against oral candidosis is concerned, both secretory IgA and cellular immunity might play a role in the protection of the oral mucosal surfaces against candidal infection. Indeed, a markedly increased prevalence of candidal infection can be seen in IgA-deficient individuals. Among patients with chronic mucocutaneous candidosis, which is a systemic disease with widespread chronic hyperplastic candidosis lesions, over 50% appear to have reduced IgA antibodies (Lehner *et al.*, 1972b).

Specific antibodies of IgG and IgM types against whole cell or antigens of *Candida* are found in most people (Winner, 1955; Lehner *et al.*, 1972a), even in those who carry *Candida* only as a commensal in their mouths or other mucosal surfaces, such as the vagina. However, serum antibodies against whole cells of *Candida* appear to vary between patients with different types of candidosis (Lehner, 1971). In the latter study, it was shown that antibody titers in chronic hyperplastic candidosis patients were not as high as in patients with *Candida*-associated den-

TABLE 3
Non-specific Factors in the Defense against Candidal Infections

Non-specific Factors	Possible Mechanisms
Skin and mucous membranes	Shedding of epithelial cell; antibiotic peptides (defensins)?
Secretions	Salivary flushing action
Antimicrobial factors in secretions	Histatins, lactoferrin, lactoperoxidase system, lysozyme
Commensal bacteria	Inhibition of candidal colonization
Phagocytosis	Polymorphonuclear leukocytes and macrophages
Natural killer cells	Direct cytotoxicity
Complement binding	Inhibition of complement activation

ture stomatitis. It had been suggested that this observation perhaps reflects the greater mucosal area involved in the latter condition with the concurrent transmucosal penetration of antigens compared with most cases of chronic hyperplastic candidosis.

Serum antibodies are generally not capable of killing *Candida*, even in concert with complement. However, they appear to act as opsonins for polymorphonuclear leukocytes and macrophages. They are also believed to act as chemotactic agents for these cells, thereby attracting them to the site of infection. As with other infections, the fixation of complement by antibody leads to the release of C3a and C5a, which are chemotactic (Challacombe, 1990).

Lehner (1966) showed, using immunofluorescent assays, that salivary antibody titers were raised in patients with candidosis, although not stated specifically as candidal leukoplakia, compared with carriers and non-infected controls. Epstein *et al.* (1982) confirmed this, using similar techniques, by demonstrating that the rise in titer of both IgG and IgA antibodies, mainly the IgA antibodies, was able to inhibit the adherence of *Candida albicans* to buccal epithelial cells.

Budtz-Jørgensen (1973) demonstrated the role of cellular immunity in resisting chronic candidal infections in the rhesus monkey. Evidence for the significant role played by cellular immunity against *Candida* infection can be found in the observation that infection by the fungus is a widespread problem among patients with severe T-cell defects and not in those with B-cell defects, unless the latter also have concomitant T-cell defects (Challacombe, 1994). Williams *et al.* (1997), who characterized the inflammatory cell infiltrate in CHC using immunocytochemical techniques, concluded, on the basis of their findings (*vide infra*), that mucosal defense against *Candida* infection involves a cell-mediated reaction. This consists of recruitment of macrophages and local production of immunoglobulin with a prominent IgA component.

Finally, in another study on the role of Langerhans cells in candidosis, Daniels *et al.* (1985) found them distributed in a fairly patchy manner, whereas control tissue sections showed even distribution. The difference in the numbers of cells, however, was not statistically significant. Although candidal antigens themselves were not detectable, these workers found ATPase-positive Langerhans cells among, or at least near, intra-epithelial hyphae and T6-positive cells separated from the hyphae by epithelial cells. They postulated that this difference in distribution of ATPase-positive and T6-positive Langerhans cells may indicate locations of two cell subtype, or a change in T6 antigen expression by the Langerhans cells closest to the candidal hyphae.

(2.3.3) Nutritional factors in the pathogenesis of candidal leukoplakia

Samaranayake (1986) has reviewed the extensive literature on the role of nutritional factors in the pathogenesis of oral candidosis. A summary of the available data on the role of iron deficiency shows that oral candidosis may be caused in the deficient individual by at least four mechanisms that render the oral mucosa susceptible to infection by the fungus. Iron deficiency can cause epithelial abnormalities such as hyperkeratosis and atrophy through alterations in the kinetics of the rapidly dividing cells of the oral mucosa, which, in turn, result from an impairment of iron-dependent enzyme systems (Jacobs, 1961; Rennie *et al.*, 1984; Ranasinghe *et al.*, 1989). Iron deficiency has also been shown to cause depression of cell-mediated immunity both in vivo and in vitro (Joynson *et al.*, 1972) and may also cause defects in phagocytosis and inadequate antibody production (Wilton and Lehner, 1981). Jenkins *et al.* (1977) have shown that a significant proportion of patients with chronic hyperplastic candidosis suffered from a deficiency in folic acid. Challacombe (1986) showed significant hematological abnormalities in patients with non-ulcerative diseases of the oral mucosa, including leukoplakia. Deficiencies of Vitamins A (Montes *et al.*, 1973) and B1 and B2 (De Greciansky *et al.*, 1957) are generally implicated in the causation of oral candidosis on the basis of animal experiments. There are also isolated reports of a link between deficiencies of Vitamins C and K and zinc and the presence of oral candidosis (Samaranayake, 1986).

It is probable that deficiencies in the above-mentioned micronutrients act not only alone but also in concert, through their direct effect on the nutrition and kinetics of the oral epithelium as well as the systemic effects they may cause. Carbohydrate-rich diets are particularly implicated in oral candidal infections, although not necessarily in relation to chronic hyperplastic candidosis.

(2.3.4) Blood group antigen secretor status as risk factor

ABO blood group antigens, which are essential components of red blood cells, are secreted in saliva and other body fluids of most individuals, who are thus referred to as secretors (Mourant *et al.*, 1978). These antigens are also found in all human tissues, including epithelial cells (Bird and Tovey, 1982). It is already an established fact that the secretion of these antigens reduces susceptibility to many infections (Cruz-Coke *et al.*, 1965; Barua and Pagvio, 1977; Kinane *et al.*, 1983). May *et al.* (1986) were the first to report that, when compared with the saliva of non-secretors, saliva from secretors reduced the adhesion of *C. albicans* to buccal epithelial cells. They postulated that the lectin-like adhesins found in the yeast cell wall, which would otherwise bind with receptors on buccal epithelial cells, are prevented from doing so by the blood group antigens secreted in saliva by binding to the receptors on the yeast as well as to the host cells.

Burford-Mason *et al.* (1988) subsequently demonstrated that non-secretion of blood group substances in saliva significantly increases oral candidal carriage. The same authors also showed that the blood group H antigen functions as a *C. albicans* receptor, and therefore individuals who are of the blood group O (who possess a large amount of H antigen on their cell surfaces) are very susceptible to candidal colonization and subsequent infection.

The relationship between the inherited ability to secrete blood group antigens in saliva and chronic hyperplastic candidosis was investigated by Lamey *et al.* (1991). The proportion of non-secretors of blood group antigens was significantly higher in patients with chronic hyperplastic candidosis (68%) compared with control subjects (38%). On this basis, Lamey *et al.* (1994) concluded that the inability of an individual to secrete blood group antigens in saliva might be a risk factor in the development, or persistence, of chronic hyperplastic candidosis, which some workers consider as a pre-cancerous lesion (Roed-Petersen and Pindborg, 1973; Bánóczy, 1977).

(3) Clinical Features of Chronic Hyperplastic Candidosis

CHC presenting as leukoplakia appears as well-demarcated, palpable, raised lesions that may vary from small translucent whitish areas to large opaque plaques that cannot be rubbed off. Some or all areas of the plaque may have a smooth, homogenously white surface, and if this feature predominates, the lesion is referred to as a homogenous leukoplakia. However, the surface often has erythematous areas intermingled with white areas that, more often than not, possess a nodular characteristic. Such lesions are referred to as nodular or speckled leukoplakia. It was Pindborg *et al.* (1963) who pioneered the concept of nodular (speckled) leukoplakia, based mostly on their findings in the labial commissure. They found 35 nodular leukoplakias in a sample of 185 mostly commissural leukoplakias (18.9%). Pindborg (1980) deplored the tendency among certain workers to consider candidal leukoplakias as florid oral papillomatosis, or Bowen's disease (Nater *et al.*, 1977). Although many homogenous lesions are asymptomatic, speckled leukoplakias cause intermittent soreness or discomfort.

As mentioned earlier, the most common site for these lesions is the buccal mucosa, especially the commissural areas (Fig. 1). The palate and tongue may also be involved, although less frequently, with the former being affected relatively more often. Not uncommonly, the commissural lesions of CL tend to be associated with angular cheilitis. Indeed, in about one-third of candidal leukoplakias, other forms of oral candidosis are found to coexist (Arendorf *et al.*, 1983). These are *Candida*-associated denture stomatitis, angular cheilitis, median rhomboid glossitis (MRG), and an oval or circular erythematous lesion on the palate in the area corresponding to that of MRG. The term "chronic multifocal candidosis" has been used to describe this tetrad, the constant member of the group being commissural candidal leukoplakia (Cernea *et al.*,1965; Holmstrup and Besserman, 1983).

(4) Pathology

(4.1) Histopathology of chronic hyperplastic candidosis

The histopathology of chronic hyperplastic candidosis was first described by Cawson (1966a) and Cawson and Lehner (1968). The features of the lesion may vary according to the clinical subtype, whether homogenous or speckled, and the degree of dysplasia present in the lesion. Homogenous leukoplakia may be either hyperorthokeratinized or hyperparakeratinized (Pindborg, 1980). Epithelial dysplasia is generally absent in homogenous leukoplakia but is more commonly noted in nodular leukoplakias. Various degrees of a chronic inflammatory cell infiltrate are seen in the lamina propria; the parakeratinized surface epithelium may show irregular separation. *Candida*l hyphae may be seen invading the epithelium at right angles to the surface. In some cases, the organisms may be so sparse that several periodic acid-Schiff (PAS)-stained specimens have to be examined for candidal hyphae to be detected.

Nodular leukoplakia may show marked variations in the thickness of the epithelium. Parakeratosis is invariably present, but hyperkeratosis is rarely seen in the infected part of the specimen. The parakeratotic layer is of variable thickness, sometimes about 12 or more cells deep, generally corresponding to the depth of invasion of the hyphae. Thus, the candidal invasion always stops short of penetrating beyond the junction between the parakeratotic layer and the stratum spinosum (Cawson and Lehner, 1968). Interestingly, the reasons for this abrupt cessation of hyphal invasion beyond the so-called "glycogen-rich" cells of the epithelium remains unknown. It is likely that the cellular defenses of the host invariably overcome the hyphal penetration at this juncture. Yet, full-thickness invasion of the epithelium is not seen in compromised patients totally devoid of cellular defenses, such as those suffering from AIDS.

Another characteristic histological feature of CHC is the collections of polymorphonuclear leukocytes forming "microabscesses" associated with candidal hyphae. Indeed these are considered to be diagnostic markers. The stratum spinosum itself shows acanthosis with hyperplasia of the rete ridges.

Jepsen and Winther (1965) isolated yeasts from smears of 34% of homogenous leukoplakias and *C. albicans* alone from 91% of speckled leukoplakias. These authors observed aggregates of candidal hyphae only in smears from the latter. This finding of a strong association between candidal invasion and speckled leukoplakia was confirmed by several subsequent publications (Renstrup, 1970; Roed-Petersen *et al.*, 1970; Daftary *et al.*, 1972; Bánóczy, 1982).

(4.2) The role of *Candida* in the causation of chronic hyperplasia and epithelial dysplasia

(4.2.1) Chronic hyperplasia

Jepsen and Winther (1965) suggested that when candidal hyphae are seen in association with epithelial hyperplasia, it is due to candidal invasion of an existing hyperplastic lesion rather than to *Candida* directly causing the epithelial hyperplasia. In an attempt to refute this hypothesis, Cawson (1973) elegantly demonstrated, using a chick chorioallantoic membrane model, that *Candida albicans* is indeed able to elicit hyperplasia. Several subsequent studies have confirmed that if *Candida* does begin to invade the superficial layers of the oral mucosa, a hyperplastic response of the epithelium ensues (Sohnle and Kirkpatrick, 1978; Odds, 1988). This has been further confirmed by Jennings and MacDonald (1990), who observed a 66% increase in the average epithelial thickness in 10 patients with chronic atrophic candidosis when compared with a normal population.

Partridge *et al.* (1971) contend that such a hyperplastic response, resulting in the formation of a plaque, is directly related to the virulence of the *Candida* isolates. Corroborative

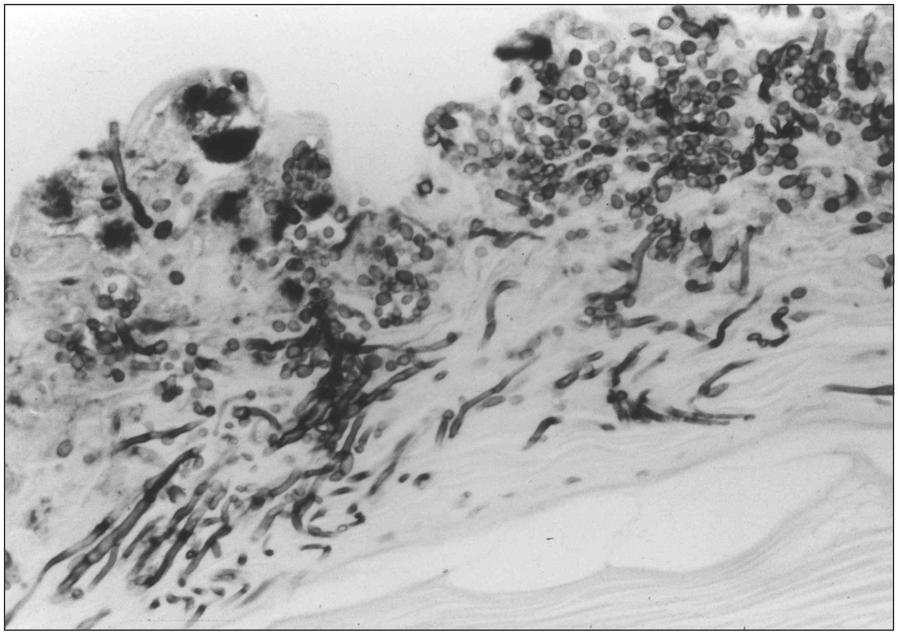

Figure 2. Grocott's methenamine silver-stained histopathological section of hyperplastic candidosis. This stain clearly demonstrates the blastospores and filamentous fungal elements (in black), although it poorly visualizes the inflammatory exudates and other host cells (x200).

data in Wistar rats were obtained by Shakir *et al.* (1986), who demonstrated that an acrylic appliance laced with *Candida* induced histological changes, including increased mitotic activity and thickening of the palatal epithelium.

Citing clinical data, Samaranayake (1990) surmised that the regression of a significant proportion of chronic hyperplastic lesions as a result of antifungal therapy is an indication that hyperplasia is a protective response of the host mucosa against a disseminated infection by *Candida*. Nagai *et al.* (1992) have presented ultrastructural evidence to support this view. In biopsies of CL, these authors demonstrated, ultrastructurally, the presence of numerous small desmosomes and the interdigitation of cytoplasmic membranes between spinous cells in both epithelial hyperplasia and epithelial dysplasia. Moreover, eosinophilic spinous cells, observed predominantly in epithelial hyperplasia, showed an intricate arrangement of dense tonofibrils. They suggested that the findings indicate provision of mechanical strength between spinous cells in *Candida*-infested oral mucous epithelium and conclude that the hyperplastic reaction is a protective response.

(4.2.2) The role of *Candida* in the induction of epithelial dysplasia

According to several authors, *Candida* infection not only causes epithelial hyperplasia but may also induce epithelial atypia, leading to malignant change. Renstrup (1970) reported epithelial atypia in 71% cases of speckled leukoplakia, with 40% of all candidal leukoplakias demonstrating epithelial atypia. In animal models, *Candida* infection can induce epithelial dysplasia when inoculated into non-dysplastic hyperplastic lesions (Zhang *et al.*, 1994).

Barrett *et al.* (1998) reviewed 223 biopsies and reported that there is a statistically significant positive association between fungal infection and moderate and severe epithelial dysplasia. An analysis of subsequent biopsies showed that epithelial dysplasia associated with fungal infection significantly worsened over time in comparison with non-infected epithelial dysplasias. In a very recent study, McCullough *et al.* (2002) (using the oral rinse technique) also described a significant correlation between epithelial dysplasia and the overall degree of oral yeast carriage in 223 patients who underwent biopsy sampling for investigations of an oral mucosal lesion. Intriguingly, in 44.6% of patients who had a histopathological diagnosis of either epithelial dysplasia or oral squamous cell carcinoma, the frequency of oral yeast carriage was significantly greater ($p < 0.001$) than that in those without such histopatholgically demonstrable lesions. The foregoing, along with the clinical evidence, suggests that up to 15% of the CHC lesions may progress into dysplastic lesions (Samaranayake and MacFarlane, 1990) and underscores the importance of close monitoring of recalcitrant lesions that do not resolve after appropriate therapy.

The probable role of yeast in oral carcinogenesis is unclear. It could be argued that the increased colonization and infestation associated with dysplasia are entirely coincidental, reflecting a change in the environmental conditions conducive to the proliferation of these ubiquitous commensals. If chronic candidal infestation were to be an important co-factor in carcinogenesis, then many more patients with chronic mucocutaneous candidiasis syndromes should develop oral carcinomas. Perhaps the mystery may lie in the genotypic attributes of the colonizing yeasts, which may be more virulent, in this respect, than their counterparts. Further research is warranted on both the phenotypes and the genotypes of species of *Candida* isolated from patients with and without oral dyspasias or carcinomas.

(4.3) Detection of *Candida* in biopsy specimens —PAS and GMS techniques

Histopathological examination of a suspected lesion is essential for the diagnosis of CHC. Because candidal hyphae tend to be poorly stained by the routine hematoxylin/eosin stain, there is a risk that they may not be adequately visualized. Therefore, special staining techniques may be required to demonstrate their presence. The periodic acid-Schiff (PAS) and Gridley's or Grocott's methenamine silver (GMS) stains are suitable for the demonstration of fungal elements within tissues, which are dyed deeply by these stains. In the latter techniques, the adjacent hydroxyl groups of the complex polysaccharides of the yeast cell wall are oxidized to aldehydes in the presence of chromic or periodic acid. In the Gridley's and PAS techniques, the aldehydes react with the Schiff reagent, and the fungi then appear a pinkish red, whereas in the GMS method the aldehydes reduce the methenamine silver nitrate complex, producing a brown-black staining as a result of the deposition of reduced silver (Fig. 2).

The presence of blastospores and hyphae or pseudohyphae may enable the histopathologist to identify the fungus as a species of *Candida* and, given the presence of other histopathological features, make a diagnosis of chronic hyperplastic candidosis. However, speciation of *Candida* in chronic hyperplastic candidosis would require cultural studies to complement the biopsy procedure, although molecular biology techniques with PCR tools have recently helped speciate *Candida* directly from biopsy specimens in archival, formalin-fixed, paraffin-wax-embedded oral mucosal tissues, by means of ribosomal DNA sequencing (Williams *et al.*, 1995, 1996).

It is important that a swab of the lesion and a smear be taken prior to the biopsy procedure. It is also important that the biopsy site be an area that has not been previously swabbed or scraped, since fungal elements may be absent from such regions, thus reducing the usefulness of the procedure (Silverman *et al.*, 1990).

(4.4) Characterization of the inflammatory cell infiltrate in candidal leukoplakia

Williams *et al.* (1997) characterized the inflammatory cell infiltrate in biopsy material of CHC using immunocytochemical techniques to stain different cellular antigens, immunoglobulins, and lysozyme. They found that the density of the infiltrate varied considerably between cases, and that the lymphocytes were predominantly T-cells (53.9%), with macrophages constituting about 14% and the B-cells 8.2%. They also found, overall, that about 61% cells contained immunoglobulins. A high proportion of cells (36.7%) contained IgA, and a smaller proportion IgM (2.5%).

(4.5) Immunocytochemical detection of *Candida* in formalin-fixed histopathological specimens

Although the detection of *Candida* in tissues, by direct and indirect immunofluorescent and ELISA techniques, was possible for several years after Lehner (1966) pioneered the application of these techniques, these methods failed to find wider use. Indeed, Silverman *et al.* (1990) questioned the usefulness of such techniques when relatively simpler and more sensitive methods were available. Furthermore, these immunofluorescent methods required frozen specimens and were not feasible with formalin-fixed tissue specimens. In recent years, with the advent of sophisticated immunocytochemical techniques involving mono- and polyclonal antibodies, it is now feasible to detect *Candida* in formalin-fixed specimens. Williams *et al.* (1998) have demonstrated the usefulness of this technique using a commercially available monoclonal antibody to detect the presence of *Candida albicans* in formalin-fixed biopsy material.

(5) **Candida** *and Oncogenesis*

(5.1) Purported role of *Candida* in oral carcinogenesis; *Candida albicans* and the carcinogenic nitrosamines

There are several reports of carcinomas developing in candidal leukoplakias or similar lesions (Robinson and Tasker, 1947: Kugelman *et al.*, 1963; Cawson, 1966a; Williamson, 1969; Eyre and Nally, 1971; Hornstein *et al.*, 1979; Cawson and Binnie, 1980). These reports claimed that candidal infection was the direct cause of the malignant transformation. Skeptics, however, have claimed that these reports do not necessarily prove a causal relationship, but merely an association, between chronic candidosis and carcinoma. It was not clear whether the host cell proliferation, secondary to candidal invasion, leads to uncontrolled epithelial growth or whether these events, together with other as-yet-unidentified factors, initiate a neoplastic change (Pindborg *et al.*, 1980).

In an early study, Russell and Jones (1975) found that the consistent colonization of the lingual mucosa of rats by artificial inoculation of candidal organisms could lead to a histological appearance similar to that of candidal leukoplakia in humans. They also observed that long-term *Candida* infection of the rat tongue resulted in epithelial hyperplasia and some features of epithelial dysplasia, but further progression to carcinoma *in situ* or squamous cell carcinoma did not take place. In a related study, Shakir *et al.* (1986) found that fungal infestation of the oral cavity leads to an initial decreased mitotic activity and a subsequent sharp and significant rise in mitotic activity. At about the same time, Franklin and Martin (1986) showed that painting the hamster cheek pouch mucosa with 50% (v/v) turpentine and liquid paraffin, followed by *Candida* inoculation and suturing of the pouches, resulted in hyperplastic changes resembling those seen in human leukoplakias.

The notion of the carcinogenic potential of *Candida* has received further support from the report of Krogh *et al.* (1987b), who demonstrated that certain *Candida albicans* biotypes are capable of producing carcinogenic nitrosamine *N*-nitrosobenzylmethylamine**,** from its precursors. They also showed that strains with high nitrosation potential were associated with lesions showing more advanced precancerous changes. Jepsen *et al.* (1988) implanted nitrosamine-treated epithelial cells into the oral cavities of rats and demonstrated that the animals that received treated cells developed carcinoma within three weeks. These investigators concluded that nitrosamines produced by *Candida* appear to play a role in the causation of oral cancer. In a separate study, O'Grady and Reade (1992) showed that *Candida* can act as a promoter of oral carcinogenesis in the rat tongue when a known carcinogen, 4-nitroquinoline-1-oxide, was repeatedly applied. It is interesting to note, however, that Lacey *et al.* (1995) have ruled out a possible role for *Candida* in the pathogenesis of uterine cervical cancer.

Field *et al.* (1989), after reviewing the evidence for a role of *Candida* in oral epithelial neoplasia, postulated that nitrosamine compounds produced by *Candida* species may directly, or in concert with other chemical carcinogens, activate specific proto-oncogenes and thus initiate the development of a malignant lesion. They further suggested that the progression of the activated cell into a tumorigenic cell might well be linked to the amplification and over-expression of oncogenes, a phenomenon observed in many other human malignancies.

(5.2) p53 expression in candidal leukoplakic lesions

Mutations in the tumor suppressor gene p53 have been observed by many investigators in almost half of head and neck lesions, including oral leukoplakias. In one immunohistochemical study, Gao (1996) elegantly demonstrated p53 expression in all 17 CL (100%) cases compared with 78% of the controls. They found that the number of p53-positive cells was significantly higher in lesions exhibiting epithelial dysplasia than in those without dysplasia ($P < 0.01$) and concluded that p53 expression may be considered as a biological marker for CL. However, Crosthwaite *et al.* (1996) reported equivocal results, in that p53 expression was demonstrable only in CHC of the lip but not when the lesion involved the buccal/commissural

TABLE 4
***Candida* Isolation in the Clinic and Quantification from Oral Samples**

Method	Main Steps	Advantages	Disadvantages
Smear	scraping, smearing directly onto slide	simple and quick	low sensitivity
Swab	taken by rubbing cotton-tipped swabs over lesional tissue	relatively simple	selecting sampling sites critical
Imprint culture	sterile plastic foam pads dipped into Sabouraud's (Sab) broth, placed on lesion for 60 sec; pad pressed on Sab agar plate and incubated; colony-counter used	sensitive and reliable; can discriminate between infected and carrier states	Reading above 50 cfu/cm^2 can be inaccurate; selection of sites difficult if no clinical signs present
Impression culture	Maxillary and mandibular alginate impressions; casting in agar fortified with Sab broth; incubation	useful to determine relative distributions of the yeasts on oral surfaces	useful mostly as a research tool
Salivary culture	Patient expectorates 2 mL saliva into sterile container; vibration; culture on Sab agar by spiral plating; counting	as useful as imprint culture	considerable chair-side time; not useful for xerostomics; cannot identify site of infection
Oral rinse	Subject rinses for 60 sec with PBS at pH 7.2, 0.1 M, and returns it to the original container; concentrated by centrifugation; cultured and counted as in previous methods	comparable in sensitivity with imprint method; better results if cfu > 50/cm^2; simple method	Recommended for surveillance cultures in the absence of focal lesions; cannot identify site of infection

mucosa, which are usually shielded from exposure to sunlight. Warnakulasuriya (2000), in contrast, asserts that p53 overexpression in oral leukoplakia has been misinterpreted as a marker of impending malignant transformation. The latter author emphasizes that, when used as a single marker, p53 is unsuitable as a predictor of malignant transformation of oral pre-cancerous lesions, and further, that there is no method sufficiently superior to conventional histopathology for the prediction of the behavior of oral pre-cancerous lesions.

(6) Mycology of Candida *in Candidal Leukoplakia*

(6.1) *CANDIDA* ISOLATION IN THE CLINIC AND IDENTIFICATION OF YEASTS FROM ORAL SAMPLES

The laboratory identification of *Candida* species is an important requirement for the diagnosis of oral candidosis in general, and this applies to CHC as well. Identification of the yeast in histopathological specimens is discussed elsewhere in this review. However, identification from oral samples obtained from lesions other than by biopsy is also necessary, particularly in epidemiological studies. Recently, Williams and Lewis (2000) and Williams *et al.* (2000) have reviewed this subject, and diagnostic methods that are available are listed in Table 4.

(6.2) IDENTIFICATION OF *CANDIDA* SPECIES

It has been shown by several workers that *C. albicans* is the predominant species associated with CL. Jepsen and Winther (1965) isolated *C. albicans* alone in 91% of speckled leukoplakias. Roed-Petersen *et al.* (1970) succeeded in culturing *C. albicans* from all leukoplakias in which hyphae were detected. Lipperheide *et al.* (1996) showed that *C. albicans* constituted 76% of the yeast isolated from oral leukoplakias.

Krogh *et al.* (1987a), who found association of *C. albicans* with 82% of leukoplakic lesions, identified the following species of yeasts as well: *Candida tropicalis*, *Candida pintolopesii*, *Candida glabrata*, and *Saccharomyces cerevisiae.* A study by Bartie *et al.* (2001), referred to in detail below, has shown the association of *C. dubliniensis* in three patients among 17 cases of CHC.

In view of the possibility that specific sub-strains of *Candida albicans* may be associated with CHC, Krogh *et al.* (1987b) biotyped the *C. albicans* isolates from these lesions and found 18 biotypes, with the most frequently occurring biotypes being 355 and 177. More interestingly, the same workers claimed that nodular leukoplakias were found to be associated with the rarely encountered biotypes 145, 175, and 575. However, other recent studies with DNA fingerprinting have cast doubt on this assertion (Lischewski *et al.*, 1999).

(6.3) STRAIN DIFFERENTIATION WITHIN *CANDIDA* SPECIES

The advent of genotyping methods based on analysis of nucleic acid sequences has made it possible for the strains involved in CHC to be identified more accurately than ever before. (For a recent review, see Dasanayake and Samaranayake, 2003.) In a recent study, Bartie *et al.* (2001), who genotyped *C. albicans* strains from patients with CHC and other disease variants using the random amplification of polymorphic DNA (RAPD) technique, demonstrated that the CHC strains are not restricted to any individual clone or cluster of clones. This study further demonstrated the genetic similarity of isolates from clinical variants of oral candidosis, but failed to show a correlation between the genotypes identified and the clinical condition of the patient. In conclusion, these genotypic and the older phenotypic findings imply that host factors are likely to be more important in the etiology of CHC than the virulence of specific strains of *C. albicans*.

(7) Treatment and Prognosis of Chronic Hyperplastic Candidosis

(7.1) General measures to eliminate predisposing factors

Since tobacco smoking has been clearly shown to be linked to the causation of many/most leukoplakias, and to candidal leukoplakias in particular, elimination of this habit is an important step in the management of CL. This applies equally to the tobacco chewing habit that is prevalent in some parts of the world. Prostheses, particularly maxillary dentures, act as reservoirs of *Candida* and have been shown to be linked to a high prevalence of CL. Although requesting patients to abandon their dentures is not a realistic option, preventive measures such as not wearing the prosthesis at night, together with stringent denture hygiene, should be encouraged to prevent candidal colonization of the denture surface. Patients using a steroid inhaler for medical reasons should be advised to rinse their mouths or drink water soon after using the inhaler (Spector *et al.*, 1982; Ellepola and Samaranayake, 2001).

Detection of anemia and deficiencies of hematinics and vitamins by appropriate investigations must be undertaken and necessary remedies prescribed.

(7.2) Specific treatment of chronic hyperplastic candidosis/candidal leukoplakia

Various modalities of treatment for CL have been used and diverse claims made regarding their success. These include medical management in the form of antifungal therapy or topical application of retinoids, bleomycin, beta carotene or mixed tea, and surgical methods (Lodi *et al.*, 2002).

Surgical methods that include cold-knife surgery, laser therapy, and cryosurgery have been in use for the treatment of oral leukoplakias. The lesion is usually excised and the wound closed, primarily if it is small, and with a split-skin graft in the case of an extensive lesion. This may or may not be combined with carbon dioxide laser therapy or cryotherapy. Many clinicians prefer to treat the lesions with a period of topical and/or systemic antifungal therapy prior to embarking on surgical management. There is a paucity of information or indeed any controlled clinical trials with regard to these management methods and no overall clinical consensus as to the best approach.

(7.2.1) Antifungal therapy

Cawson and Lehner (1968), in their first report on CL, claimed improvement and disappearance of a significant number of their cases of leukoplakia with polyene-nystatin (tablets) dissolved slowly in the mouth. Lamey *et al.* (1989) reported a case of CL with a significant degree of epithelial dysplasia that resolved within 11 days of systemic treatment with the triazole antifungal, fluconazole. Holmstrup and Bessermann (1983) reported that, following antifungal therapy, nodular lesions of the commissural areas showed the highest recurrence rate after 12 months.

(7.2.2) Medical, surgical, laser, and cryo-therapies

The available information on evaluation of the different forms of treatment of oral leukoplakia is not specific to candidal leukoplakia. However, it is useful to review the successes and failures of the different methods as they are applied to oral leukoplakias in general. Lodi *et al.* (2001) have reviewed a large number of publications on the different methods of treatment of oral leukoplakia. They analyzed reports of randomized clinical trials related to topical application of vitamin A, retinoids, bleomycin, mixed tea, or beta-carotene, individually. However, none of these treatment methods showed any significant benefit with regard to reduction of malignant transformation rates in comparison with the placebo treatment. Nevertheless, Vitamin A/retinoids and beta-carotene were associated with better rates of clinical remission. They concluded that none of the available methods could be considered as very effective. They found that there is a dearth of randomized controlled trials that have assessed the efficacy of surgical methods in the management of candidal leukoplakia.

Schoelch *et al.* (1999) analyzed the efficacy of laser treatment in the management of oral leukoplakias and claimed successful control of the lesions in 48 of 70 total cases.

Saito *et al.* (2001) have studied the effectiveness of surgical methods of treatment of oral leukoplakia in a controlled trial involving a total of 142 patients. They found that malignant transformation rates were lower in patients who underwent conventional surgical treatment *vs.* those subjected to cryotherapy.

(8) Conclusions and Future Directions

It would appear that during the nearly three and a half decades since Cawson and Lehner (1968) introduced the terms "candidal leukoplakia" and "chronic hyperplastic candidosis", the subject has attracted only sporadic attention from researchers. There appears to be some revival of recent interest, particularly in the molecular biologic features of the disease, especially in relation to diagnostics. Nevertheless, it is clear that, at present, the overall database on the etiology, pathogenesis, and management of the disease is still in its infancy. The characteristics of *Candida albicans* that enable it to produce epithelial dysplasia and eventually malignant transformation still remain far from thoroughly elucidated. The ultrastructural biochemical and molecular features of the invasive phase of the organism in relation to the hyperplastic response it evokes, and the inter- and intra-species differences that might exist, and the related yeast biology are aspects worthy of further study.

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