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Oral Candidiasis: Clinical Manifestations and Cellular Adaptive Host Responses

Robert B. Ashman and Camile S. Farah

School of Dentistry, University of Queensland, Brisbane, Queensland, Australia.

1. Introduction

Many *Candida* species are commensals of the oral mucosa. They are usually innocuous, but when conditions are favorable, they can proliferate and cause disease. *Candida* spp. are readily isolated from various sites within the oral cavity, but simple carriage does not inevitably result in the development of clinical symptoms. Whether the organism remains as a commensal, or proliferates and causes disease, is usually determined by preexisting or concomitant alterations in the integrity of the immune system.

Oral candidiasis is seen most frequently in patients at-risk, such as those suffering from HIV/acquired immunodeficiency syndrome (AIDS), immunosuppression, diabetes, xerostomia, or those wearing dentures (Fotos and Hellstein, 1992); however, clinical observations in these and other patient groups have suggested that these predisposing factors are almost invariably associated with deficiencies in innate and adaptive immune functions that compromise effective host responses against the infection. The seminal observations by Kirkpatrick and colleagues (1971) on children with chronic mucocutaneous candidiasis (CMC) strongly implicated defects in cell-mediated immunity as a major predisposing factor, and the importance of T lymphocytes, and $CD4^+$ cells in particular, has been confirmed by the high incidence of oral candidiasis in HIV-infected individuals whose $CD4^+$ lymphocyte counts are declining.

In this chapter, the epidemiology of the disease, associated risk factors, and clinical features are briefly outlined. This is followed by reviews of the host immune factors relevant to recovery from oral infection in humans and mice, and the discussion concludes with an evaluation of the ways in which these immune mechanisms may relate to the clinical manifestations of the disease.

2. Epidemiology of Oral Candidiasis

2.1. *Candida* Species

Candidiasis is most commonly caused by the yeast *C. albicans*, and to a far lesser extent by *C. glabrata*, *C. krusei*, *C. tropicalis*, *C. pseudotropicalis*, *C. parapsilosis*, and *C. guilliermondi* (Odds, 1988). More recently, oral candidiasis in HIV-positive individuals has been associated with *C. dubliniensis* (Sullivan et al., 1995).

C. albicans is a commensal residing in the oral cavity of the majority of healthy persons (Odds, 1988). It is a dimorphic fungus that can exist both in a yeast phase (blastospore, blastoconidial) and a hyphal (mycelial) phase. Depending on the environmental conditions, they may develop either in the mycelial form, composed of long branching septae or filaments, or as spherical or ovoid yeast cells. In the yeast phase *C. albicans* are from 2–8 to 3–14 μm in size, but hyphae can extend a few hundred micrometers (Saltarelli, 1989). They reproduce by multilateral budding and do not undergo a sexual cycle. Dimorphism is relevant both to the pathogenicity of the yeast and to the clinical problems of diagnosis and treatment (Saltarelli, 1989).

C. albicans is the most common *Candida* species isolated from the oral cavity both in the healthy and the diseased (Samaranayake and MacFarlane, 1990). Symptom-free oral carriage of *Candida* organisms has been recognized for many years, with the reported prevalence ranging from 3% to 48% in clinically normal mouths of healthy adults (Arendorf and Walker, 1980), and 45% to 65% in healthy children (Odds, 1988). The oral carriage of yeasts is higher in hospitalized than ambulant patients, with a median carriage rate of 54.7% for all species and 38.1% for *C. albicans* alone (Wilkieson et al., 1991).

In the past *C. dubliniensis* was probably identified as an atypical strain of *C. albicans*. Although it was initially found to colonize the oral cavity and to be a cause of oropharyngeal candidiasis (OPC) in HIV-positive patients (Sullivan et al., 1995), it has since been found to colonize and infect various other groups (Redding, 2001; Kantarcioglu and Yucel, 2002; Tekeli et al., 2002). Incidence rates for *C. dubliniensis* in oral cultures from adults vary from 3% in asymptomatic HIV-negative individuals to 32% in HIV-positive patients with clinical signs and symptoms of OPC (Coleman et al., 1997; Meiller et al., 1999; Fisher et al., 2001; Giammanco et al., 2002). *C. dubliniensis* has also been detected in pediatric patients (Sano et al., 2000), with one study reporting positive cultures in 11% of HIV-positive pediatric patients (Brown et al., 2000). Mixed infections, especially with *C. albicans*, are common in patients who are positive for oral *C. dubliniensis* (Schorling et al., 2000).

C. glabrata is much less pathogenic than *C. albicans*, but in recent years it has emerged as a significant pathogen in humans. It is currently the second to third leading cause of candidiasis of all kinds and comprises approximately 5% of all *Candida* isolates (Thuraisingam and Denning, 2000). The role of *C. glabrata* in OPC is controversial, and the yeast is mostly seen in cases of systemic candidiasis. *C. glabrata* is commonly isolated along with *C. albicans* when cultured from patients with OPC. *C. glabrata* makes up between 5% and 10% of all oral isolates recovered from HIV-positive patients who have OPC (Fidel et al., 1999). OPC can be caused solely by *C. glabrata* (Hoegl et al., 1998), with one study reporting that up to 14% of all oropharyngeal infections were caused by this species (Masia Canuto et al., 2000). It was estimated that approximately 30% of patients over 80 years of age were colonized orally with *C. glabrata* (Lockhart et al., 1999), but the percentage was almost doubled if the patients wore dentures.

OPC caused by *C. tropicalis* has been reported but is relatively rare. Colonization on the other hand is quite common; approximately 6% of yeast isolates other than *C. albicans* in HIV-positive patients are *C. tropicalis* (Cartledge et al., 1999). It is a pathogenic organism, especially in patients receiving chemotherapy for hematologic malignancies, and OPC associated with *C. tropicalis* can cause fungemia in these patients (Redding, 2001).

Oral colonization with *C. krusei* is common, making up approximately 20% of yeasts other than *C. albicans* cultured in HIV-positive patients (Cartledge et al., 1999), but OPC caused by *C. krusei* alone is rare.

2.2. Pathogen Risk Factors

Oral infection with *Candida* is associated with certain pathogenic variables. Adhesion of the yeast to epithelial cell walls, an important first step of infection, is promoted by fungal wall components such as mannose, C3d receptors, mannoprotein, and saccharins (Ghannoum et al., 1986; Brassart et al., 1991; Kanbe et al., 1991). Hydrophobicity (Hazen et al., 1991) and the ability to bind to host fibronectin (Klotz and Smith, 1991) are also important pathogenic mechanisms used by the yeast in the initial stages of infection. Some other factors important in host infection include germ tube formation (Sobel et al., 1984), presence of mycelia (Saltarelli et al., 1975), endotoxins (Cutler et al., 1972), and proteinases (Kwon-Chung et al., 1985).

The family of secreted aspartyl proteinases (SAPs) are considered to be one of the more important of the putative virulence factors of *C. albicans*. Analysis of patients with asymptomatic *Candida* carriage, and those with oral candidiasis, showed that

SAP2 and SAPs 4 to 6 were expressed in both subpopulations, whereas SAP1 and SAP3 were observed only in patients with oral disease (Naglik et al., 1999). More extensive studies (Naglik et al., 2003a) confirmed that SAP2 and SAP5 were the most common genes expressed during both infection and carriage, whereas expression of SAP1, SAP3, SAP4, SAP7, and SAP8 correlated with oral disease. Using an in vitro model, the gene products of SAPs1 to 3, but not SAPs4 to 6, appeared to be predominantly responsible for damage to reconstituted human epithelium (Schaller et al., 1999), although studies with specific SAP mutants suggested that the yeast may have the ability to compensate for the loss of one SAP gene by the upregulation of others.

Interestingly, viable, but not heat-killed *Candida*, was able to induce increased expression of genes coding for a variety of immunologically relevant cytokines and chemokines by epithelial cells (Schaller et al., 2002), and cytokine production correlated with the virulence of the organism. This suggests that the "quality" of the infectious challenge may influence the nature of the immune response elicited. This concept will be considered later, in relation to the different manifestations of oral infection.

2.3. Host Risk Factors

Although the transition from commensalism to disease may be associated with the virulence characteristics of the organism, it is widely accepted that host factors are of paramount importance in the development of the infection. *Candida* species are strictly opportunistic pathogens, which cause disease when the host defenses are defective—hence the designation "disease of the diseased" given to *Candida* infections (Trousseau, 1869).

The local intraoral environmental milieu, such as the presence of prostheses, plays a crucial role in the disease process. Indeed it is the combination of the microbial virulence factors, environmental factors, and host defense factors that determines the ultimate outcome of infection. The major local and systemic factors that predispose humans to candidiasis have been classified as natural, dietary, mechanical, and iatrogenic (Odds, 1988), but only those relevant to immune dysfunction are considered here.

Host defense mechanisms are impaired in patients with malignant disease, particularly as a consequence of chemotherapy and radiotherapy. Longitudinal studies of patients undergoing radiation therapy to the head and neck show significant increases in *Candida* species counts on the tongue surface, in whole saliva, and in dental plaque (Epstein et al., 1984; Samaranayake et al., 1988). Therapeutic interventions can also reduce numbers and impair the function of polymorphonuclear and mononuclear phagocytes, leading to oral candidiasis (Kostiala, 1986).

Chronic hyperplastic candidiasis may occur as part of CMC, often with identifiable immunologic or endocrine abnormalities as major factors. Endocrine disorders such as hypothyroidism, hypoparathyroidism, and adrenal insufficiency, have a familial incidence and are found in children and young adults, particularly in girls. The most frequently associated endocrine manifestations include idiopathic hyperparathyroidism and hypoadrenocorticism, but candidiasis follows only where there is an immune defect (Kostiala et al., 1979).

Fungal infections, particularly atrophic and pseudomembranous candidiasis, are common in patients with HIV infection. The immunodeficiency affecting T helper lymphocytes during HIV infection makes patients with the disease more predisposed to secondary infections, notably opportunistic *Candida* infections. The first patient diagnosed with AIDS presented with oral candidiasis (Gottlieb et al., 1981a), and oral candidiasis was a common feature in patients who eventually developed AIDS (Gottlieb et al.,

1981b). Oral candidiasis occurs in 75% of HIV-positive patients (Palmer et al., 1996) and 92% of patients diagnosed with AIDS had oral candidiasis (McCarthy et al., 1991). Indeed more than 90% of patients who are HIV-positive will develop at least one episode of oral candidiasis during the progression to AIDS. The erythematous variant is most frequently seen in these patients, followed by the pseudomembranous, angular cheilitis, and then hyperplastic candidiasis (Samaranayake and MacFarlane, 1990).

A myriad of immunological abnormalities occur as a consequence of HIV infection, particularly as the disease progresses to AIDS (Fauci, 1993). Monocytes and macrophages express CD4, and HIV can directly infect these cells (Alkhatib et al., 1996). There are also other alterations to mononuclear phagocyte function including alterations in phenotypic marker expression, accessory cell function, chemotaxis, cytokine production, and respiratory burst activity (Ho et al., 1994; Trial et al., 1995; Wahl et al., 1996). Nonetheless, profound $CD4^+$ T cell depletion is the immunological hallmark of AIDS, and is the most likely factor accounting for the increased susceptibility of these patients to opportunistic infections. The role for $CD4^+$ T cells in host resistance against opportunistic fungal infections is supported by the frequent occurrence of fungal infections in patients with idiopathic $CD4^+$ T cell lymphocytopenia, a condition characterized by low $CD4^+$ counts in the absence of HIV infection (Duncan et al., 1993).

Oral candidiasis occurs in association with congenital deficiencies in both innate and adaptive immunity. Chronic recalcitrant mucocutaneous candidiasis is particularly common in patients with DiGeorge's syndrome (Cleveland et al., 1968), a condition characterized by depletion of T cells in the thymus-dependent areas of lymph nodes and in peripheral blood due to thymic hypoplasia. Patients with severe combined immunodeficiency (SCID) syndrome demonstrate multiple defects in cell-mediated immune functions, and frequently suffer from CMC that may disseminate to other tissues (Porter and Scully, 1990). Syndromes that affect innate immunity include hereditary myeloperoxidase (MPO) deficiency, in which the lack of MPO in the granules of polymorphonuclear leukocytes (PMNLs) and macrophages results in impaired killing of *C. albicans* (Lehrer and Cline, 1969; Klebanoff, 1970), and predisposes to recurrent episodes of oral thrush or CMC in these patients (Lehrer and Cline, 1969; Kirkpatrick et al., 1971). Patients with Chediak–Higashi syndrome, an autosomal recessive disease presenting with abnormal neutrophils, neutropenia, and impaired chemotaxis (Wolff, 1972; Oliver and Essner, 1975), also commonly suffer from candidal infections.

3. Clinical Manifestations of Oral Candidiasis

The classification of oral candidiasis has been fraught with difficulties and complications, due to the many manifestations the disease can take and because of the multifaceted etiology of the different conditions involved. Recently, it has been suggested that oral candidiasis can be arranged into two categories based on the distribution of the lesions (Scully et al., 1994): Category I, candidal infections confined to oral and perioral tissues (primary oral candidiasis) and Category II, disorders where oral candidiasis is a manifestation of generalized systemic mucocutaneous candidal infection (secondary oral candidiasis). Category II lesions are divided into subgroups, which take into account CMC and other immune defect disorders such as SCID syndrome, DiGeorge's syndrome, and AIDS.

The following clinical descriptions are based on revised classifications by Samaranayake and Yaacob (1990), Holmstrup and Axell (1990), and Samaranayake (1991).

3.1. Pseudomembranous Candidiasis

Pseudomembranous candidiasis is characterized by whitish-yellowish creamy patches on the surface of the oral mucosa and tongue (Fig. 4.1a). The lesions develop into confluent plaques that resemble milk curds and can be wiped off to reveal a raw erythematous base (Odds, 1988). The plaques consist of necrotic material and desquamated parakeratotic epithelium, penetrated by *C. albicans* yeast cells and hyphae that invade as far as the stratum spinosum. Edema and microabscesses containing PMNLs are found in the outer layers of the epithelium. The deeper parts of the epithelium show acant sis, and the inflammatory response in connective tissue comprises lymphocy plasma cells, and PMNL (Odds, 1988). T form of the disease is most commc found in infants, the elderly, and those ter nally ill (Finlay, 1986), particularly in con nation with severe underlying conditi such as leukemia, and HIV and AI (Samaranayake and MacFarlane, 1990).

3.2. Erythematous Candidiasi

This condition is mainly associated v the use of corticosteroids or broad-spectr

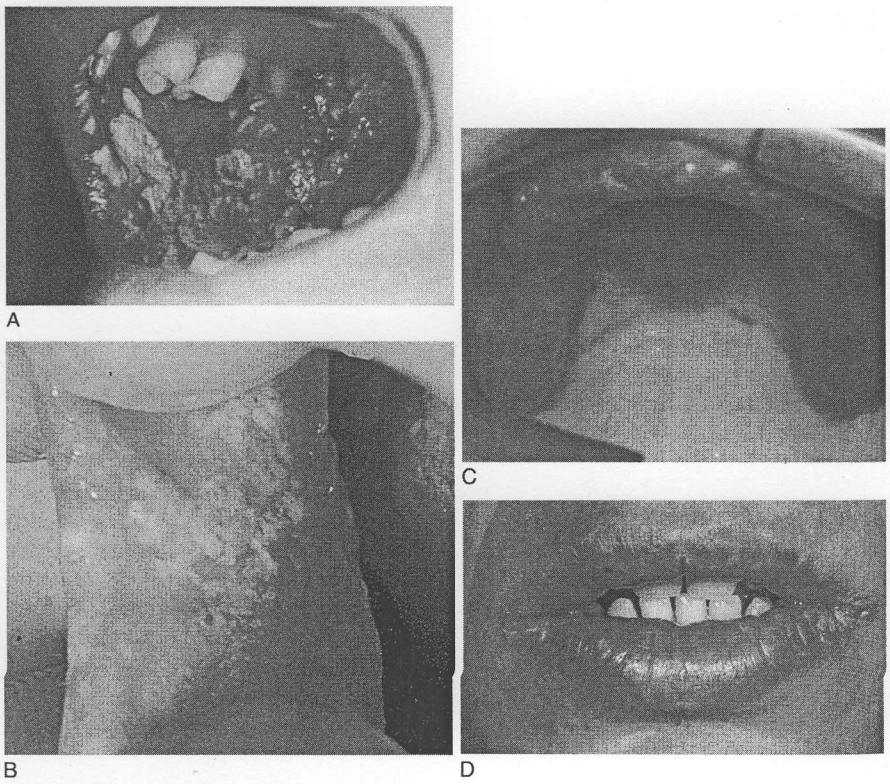

Figure 4.1. Clinical presentation of acute pseudomembranous candidiasis (A), chronic mucoc neous candidiasis (B), *Candida*-associated denture stomatitis (C), angular cheilitis (D),

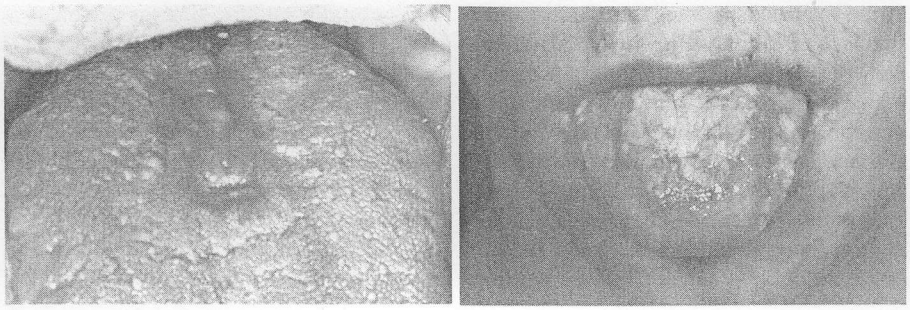

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Figure 4.1. *Cont'd* median rhomboid glossitis (E), and hyperplastic candidiasis (F). (Reprinted from publication *Clinics in Dermatology*, 18: 553–562, Farah, et al. "Oral Candidosis" © 2000 with permission from Elsevier Inc.)

antibiotics. More recently it has commonly been seen in HIV-positive patients (Dodd et al., 1991). Clinically, it is characterized by erythematous areas generally on the dorsum of the tongue, palate, or buccal mucosa. Lesions seen on the dorsum of the tongue classically present as depapillated areas (Scully et al., 1994). The condition is relatively rare, but in the acute form it is consistently painful (Lehner, 1967). The histopathology of acute erythematous candidiasis is essentially like other forms of the disease, with pseudohyphae penetrating and extending into the superficial epithelium. The inflammatory reaction is characterized by neutrophils in the epithelium and a lymphocytic infiltrate in the connective tissue.

3.3. Chronic Hyperplastic Candidiasis

Hyperplastic candidal lesions are chronic, discrete raised lesions that vary from small, palpable, translucent, whitish areas to large, dense, opaque plaques (Fig. 4.1f). The homogeneous form presents as a uniform adherent white plaque, whereas the nodular (speckled) lesion has a clinical appearance of multiple white nodules on an erythematous background (Walker and Arendorf, 1990). Neither lesion will rub off. Hyperplastic candidiasis usually occurs on the inside surface of the cheeks, palate, and tongue (Walker and Arendorf, 1990). Biopsy is important, as the condition is premalignant and shows varying degrees of dysplasia (Samaranayake and MacFarlane, 1990). Histopathological examination of the lesions reveals parakeratosis showing irregular separation and epithelial hyperplasia, with *Candida* invasion restricted to the upper layers of epithelium (Daniels et al., 1985). Polymorphonuclear microabscesses form in the epithelium beneath the candidal hyphae, with a poorly demarcated chronic inflammatory infiltrate of lymphocytes and plasma cells in the upper half of the corium. Mitotic activity is often increased, but restricted to the basal and suprabasal layers of the stratum spinosum. Epithelial dysplasia is more common in the nodular form (Walker and Arendorf, 1990).

3.4. *Candida*-associated Denture Stomatitis

Classically this condition presents as chronic erythema and edema of the

denture-bearing mucosa, especially under maxillary prostheses (Fig. 4.1c). The patient is usually symptom-free, but may complain of slight soreness and angular cheilitis can be a presenting complaint. Other factors such as bacterial accumulation, reduced salivary protection, and mechanical irritation may be implicated in denture stomatitis, which is present in approximately 50% of complete denture wearers (Budtz-Jorgensen, 1974). Histological examination of the tissues beneath the dentures shows proliferative or degenerative responses (Razek and Shaaban, 1978) with reduced keratinization and thinner epithelium (Watson and MacDonald, 1982). Tissue invasion by *Candida* does not occur as commonly as other forms of oral candidiasis, and relatively little yeast is isolated from the mucosal surface (Budtz-Jorgensen, 1974).

3.5. Angular Cheilitis

Clinically, angular cheilitis presents as sore, erythematous, fissured lesions, affecting the angles of the mouth, and is commonly associated with denture stomatitis (Fig. 4.1d) (Budtz-Jorgensen, 1974). In orofacial granulomatosis, a significant number of patients have angular cheilitis (Samaranayake, 1988), and it may also be seen in AIDS (Samaranayake and Holmstrup, 1989). As mentioned earlier, the condition can be associated with iron deficiency anemia or vitamin B_{12} deficiency (Scully and Cawson, 1998).

3.6. Median Rhomboid Glossitis

Median rhomboid glossitis is characterized by an area of papillary atrophy that is elliptical or rhomboid-like, symmetrically placed and centered at the midline of the tongue, anterior to the circumvallate papillae (Fig. 4.1e) (Walker and Arendorf, 1990). Occasionally, midline glossitis presents with a hyperplastic exophytic or lobulated appearance. Histopathologically, candidal hyphae are seen invading the superficial layers of the parakeratotic epithelium with elongated hyperplastic rete ridges extending into the corium, a PMNL infiltrate occupying the epithelium, and a lymphocyte infiltration in the corium erupting into the bases of the epithelial processes (Walker and Arendorf, 1990).

3.7. Chronic Mucocutaneous Candidiasis

CMC is a term given to a group of heterogeneous disorders that are characterized by persistent superficial candidal infection of the mouth, skin, and nail beds, sometimes producing granulomatous masses over the face and scalp (Fig. 4.1b) (Kirkpatrick et al., 1971; Edwards et al., 1978). The principal clinical features include chronic oral candidiasis, chronic cutaneous candidiasis, and chronic vulvovaginal candidiasis (Chilgren et al., 1967). Oral candidiasis has been observed in more than 90% of all CMC patients (Odds, 1988). The tongue can become enlarged, fissured, and may have hyperplastic nodules on the lateral borders. Painful angular cheilitis is frequent (Porter and Scully, 1990). CMC is associated with a variety of primary immunodeficiencies such as SCID syndrome, Nezelof's syndrome (thymic alymphoplasia), DiGeorge syndrome (congenital thymic aplasia), hyperimmunoglobulin E syndrome, MPO deficiency, and endocrine disorders, especially Addison's disease and hypoparathyroidism (Porter and Scully, 1990, 1993a,b). The oral lesions of CMC have histopathological features similar to those of chronic candidiasis (Cawson and Lehner, 1968), although candidal infection can occasionally spread into the pharynx, larynx, or esophagus, but further visceral involvement is rare (Porter and Scully, 1990).

4. Cellular Adaptive Host Responses

4.1. Clinical Studies

CMC is frequently seen in association with endocrinopathies (Kirkpatrick et al., 1971), but it is generally recognized that the main predisposing factor is a defect in cell-mediated immunity (Kirkpatrick, 1994). These patients can be grouped according to their specific abnormalities in immunological responsiveness (Kirkpatrick, 1989), but there is no *Candida*-specific defect common to all groups. Nevertheless, therapy directed at restoration of cellular immune function generally results in remission, and the demonstrable link between systemic cell-mediated immunity and mucosal defense mechanisms has been crucial in developing an understanding of the pathogenesis of the disease.

The link between CMC, immunodeficiency, and endocrinopathies is now well-recognized, but with the spread of HIV infection and AIDS, and the increasing use of aggressive chemotherapeutic regimens for transplantation and cancer patients, severe oropharyngeal infections are appearing in a much greater variety of clinical contexts. These include situations in which there is a deficiency in cell-mediated immune responses, as well as others in which the dominant feature is neutropenia. These observations suggest that host responses against oral and OPC may involve not only cells of the adaptive immune response, but also phagocytic cells, and other nonspecific innate immune effector mechanisms.

Human epithelial cells from the oral cavity have recently been shown to possess inhibitory activity against both *Candida* yeasts and hyphae (Steele et al., 2000). The anti-*Candida* activity required cell contact, and in these experiments, neither saliva nor culture supernatants alone inhibited *Candida* growth, nor was the epithelial cell activity enhanced in the presence of saliva. Although differential staining showed that the effect of the epithelial cells was candidastatic rather than candidacidal (Nomanbhoy et al., 2002), the phenomenon may be of some biological significance, as epithelial cell anti-*Candida* activity was significantly lower in HIV-positive patients with OPC (Steele et al., 2000). The oral epithelial cells may also act to enhance effector cell activity through the production of cytokines and/or chemokines that increase recruitment of phagocytic cells and stimulate innate immunity.

After challenge with *C. albicans*, primary oral mucosal epithelial cells and oral epithelial cell lines produced interleukin-8 (IL-8) (Dongari-Bagtzoglou and Kashleva, 2003b), and a proportion also produced granulocyte-macrophage colony-stimulating factor (GM-CSF) (Dongari-Bagtzoglou and Kashleva, 2003a). Both responses were dependent on physical contact with viable yeasts and were optimal when the yeast germinated into hyphae. IL-8 secretion was dependent, at least in part, on autocrine production of IL-1α (Dongari-Bagtzoglou and Kashleva, 2003b). In addition, oral fibroblasts have been shown to produce both IL-6 and IL-8 after exposure to *C. albicans* in culture (Dongari-Bagtzoglou et al., 1999). Thus, the oral mucosa may, after infection, produce a cytokine microenvironment that is able to influence the development and maturation of the cell-mediated immune response against the yeast.

It is currently believed that host responses against *C. albicans* are determined predominantly by the relative dominance of Th1-type cytokines that lead to recovery and the development of protection, or Th2-type cytokines, that are associated with susceptibility to infection (Romani, 1999). However, studies in humans have given variable results. Adult patients with chronic oral candidiasis showed significantly lower levels of serum IFN-γ healthy controls (Szkaradkiewicz et al., 1998), but there have been no more recent studies of oral candidiasis in the absence of other infections.

Analysis of salivary cytokine profiles in normal (HIV-negative) individuals showed a Th0/Th1 profile, whereas in HIV-positive individuals, it was of a predominantly Th2-type (Leigh et al., 1998), apparently as a consequence of a reduction in Th1-type rather than an enhancement of Th2-type cytokines. HIV-positive individuals with OPC tended to show a more exaggerated Th2-type salivary cytokine profile, but the relevance of this to the pathogenesis of the *Candida* infection is unclear at present. Both HIV-positive and HIV-negative patients displayed *Candida*-specific lymphocyte proliferative responses, and although skin test reactivity to *C. albicans* antigens in HIV-positive patients with low $CD4^+$ cell counts was reduced compared to controls (Leigh et al., 2001), there were no consistent differences in systemic immune responses that correlated with the development of OPC.

Denture stomatitis is another manifestation of oral infection with *C. albicans*, although the extent of colonization appears to be related to the severity of the inflammation induced by external factors, such as smoking or the wearing of dentures at night (Barbeau et al., 2003). Patients with *Candida*-associated denture stomatitis show impaired responses against antigens of *C. albicans*, as demonstrated by skin test and leukocyte migration inhibition assays (Davenport and Wilton, 1971), and may have overactive suppressor T cells or other lymphocyte/phagocyte defects (Iacopino and Wathen, 1992), indicating that normal immunoregulatory responses can also be impaired.

Nevertheless, analysis of salivary cytokines in HIV-negative individuals either with or without denture stomatitis demonstrated a mixed Th1/Th2 profile (Leigh et al., 2002), with no significant differences between the two groups, indicating that susceptibility to *Candida*-associated denture stomatitis in the immunocompetent patient was not associated with a bias towards production of Th2-type cytokines. In contrast, analysis of serum levels of interleukins and their soluble receptors found significant increases in the concentrations of IL-6 and TNF-α in both patients with dentures, and those with denture stomatitis, compared to normal controls (Pietruski et al., 2000), whereas concentrations of soluble TNF receptor were reduced in these same groups.

Phenotypic analysis by immunohistochemistry and electron microscopy of cells of the oral mucosal immune system present in biopsies of erythematous and pseudomembranous candidiasis in HIV-positive patients has shown that the superficial lamina propria and basal epithelial layer was populated by $CD1a^+$ Langerhans cells, with an infiltration of $CD8^+$ lymphocytes. $CD36^+$ dendritic macrophages and lymphocytes were detected within the submucosa, although $CD4^+$ cells were absent from the infiltrate (Romagnoli et al., 1997). In the pseudomembranous form, $CD14^+$ leukocytes were found in the basal epithelial layer. Langerhans cells were significantly more numerous in erythematous than in pseudomembranous candidiasis. It is clear that oral candidiasis is associated with perturbations in the number and state of differentiation of lymphocytes and dendritic cells, these being more severe in the pseudomembranous than in the erythematous form. These alterations may play a role in the pathogenesis and evolution of the disease (Romagnoli et al., 1997).

Further immunohistochemical evaluation of T cells in both HIV-negative and HIV-positive individuals showed a majority of $CD8^+$ rather than $CD4^+$ cells equally distributed throughout the buccal mucosa in cases where these patients were OPC-negative, irrespective of blood $CD4^+$ cell numbers. In contrast, $CD8^+$ cells in lesions from HIV-positive/OPC-positive persons were present in significantly higher numbers, and concentrated at the lamina propria–epithelium interface, a considerable distance from the *Candida* at the outer epithelium. Dual fluorescence and confocal microscopy

confirmed that the majority of $CD8^+$, but not $CD4^+$ cells, were T cells by the presence or absence, respectively, of CD3 on each cell type. These results suggest that $CD8^+$ T cells may be important for oral host defense against OPC, especially when $CD4^+$ T cell numbers are reduced (Myers et al., 2003).

Characterization of the inflammatory cell infiltrate in oral mucosal biopsy material of chronic hyperplastic candidiasis (Williams et al., 1997) showed that T lymphocytes were the dominant cell type, with fewer macrophages and B lymphocytes. Many Ig-containing cells were seen, and although IgG-containing cells predominated, there was a high proportion of IgA-containing cells with few IgM-containing cells. Many neutrophils, together with smaller numbers of T lymphocytes and macrophages, were seen in the epithelium. These authors have suggested that mucosal defense to *Candida* infection involves a cell-mediated reaction in which there is recruitment of macrophages and local production of immunoglobulin with a prominent IgA component.

4.2. Mouse Models

Mucosal candidiasis has been studied in a number of different models—gastrointestinal (GI), vaginal, and oral—and it has perhaps been too readily assumed that results obtained from infection of one particular mucosal site (e.g., the GI tract) can be extrapolated to other manifestations of the disease. It has recently become clear that this is not the case (Fidel, 2002), and that there may be significant differences in the "mix" of adaptive and innate immune responses that mediate responsiveness to *Candida* at the different mucosal sites. Nevertheless, many valuable insights have been obtained by Balish and colleagues using infection of the GI tract, and in the following discussion, attempts will be made to integrate these findings with those from the various oral models.

Models used for the study of oral candidiasis have been of three kinds: those using normal mice (Lacasse et al., 1993; Elahi et al., 2000; Farah et al., 2001a), those using immunodeficient mice (Farah et al., 2002b), and those that involve conditioning by treatment with immunosuppressive drugs (Kamai et al., 2001; Takakura et al., 2003).

4.2.1. Studies in Normal Mice

In general, the study of oral infection in normal mice has sought to identify crucial host variables by comparison of responses in genetically defined inbred mice. In inbred mice, oral candidiasis closely resembles the human disease (Farah et al., 2002b). BALB/c and DBA/2 mice are of the same major histocompatibility complex (MHC) type, but BALB/c is C5-sufficient, whereas DBA/2 is C5-deficient. Although DBA/2 mice were somewhat more prone to infection than BALB/c mice (Chakir et al., 1994), oral infection in both strains resulted in an increase in $MAC\text{-}1^+$ cells and a comparable recruitment of $CD4^+$ and $CD8^+$ T lymphocytes into the mucosal tissue. Thus, in contrast to systemic infection, complement-derived chemotactic factors appear to have little or no effect on inflammatory processes in the oral mucosa. In addition, the number of intraepithelial $CD4^+$ T cells was five- to sevenfold greater in infected animals when compared to control mice (Deslauriers et al., 1995). Using the same mouse strains, Elahi and colleagues (2000) demonstrated significantly higher *Candida*-specific proliferation by cells from the draining lymph nodes of BALB/c as compared to DBA/2 mice, after oral infection.

Although these studies in vitro strongly implicated T cells in the host response against oral infection, initial attempts to demonstrate their essential role in host resistance in normal mice by antibody depletion of CD4 or CD8 cells mice were unsuccessful. However, when the head and neck were irradiated, at a dose sufficient to

block lymphocyte proliferation, depletion of $CD4^+$, but not $CD8^+$, lymphocytes caused a prolongation of infection and increased the severity of oral lesions (Farah et al., 2001b). This was interpreted as indicating that the immune response in the oral cavity was essentially self-contained, but that $CD4^+$ cells from the systemic circulation had the potential to exert a protective effect. An alternative, but not mutually exclusive explanation, is that irradiation-induced tissue damage favored further proliferation of the yeast. This could place additional demands on the local immune response, resulting in recruitment of $CD4^+$ cells from the peripheral circulation. In the irradiated mice, recovery from infection was associated with production of high levels of IL-12 by lymph node cells, but concentrations of IFN-γ in infected mice were comparable to those in controls.

The importance of T cells and cell-mediated immunity in oral candidiasis has been supported by studies in infectious disease models. Mice infected with the Du5H(G6T2) mixture of mouse leukemia viruses develop a disease (murine AIDS or MAIDS) that exhibits many of the immune abnormalities found in human HIV infection. When these animals were orally colonized with *C. albicans*, approximately 30% developed recurring 2- to 3-week episodes of acute *Candida* proliferation, which were thought to result from virally induced fluctuations in the levels of $CD4^+$ T cells (Deslauriers et al., 1997). Similarly, transgenic mice that expressed HIV type 1 in immune cells (de Repentigny et al., 2002) showed a sustained enhancement of oral burdens of *C. albicans*, penetration by *Candida* hyphae of the stratified squamous epithelium of the oral cavity, and a mononuclear inflammatory cell infiltrate in the mucosa.

These results are generally consistent with those derived from models of orogastric candidiasis. Euthymic mice infected in this manner developed *Candida*-specific lymphoproliferative and delayed-type hypersensitivity (DTH) responses that correlated with the clearance of hyphae from mucosal surfaces (Balish et al., 1990). Conversely, depletion of $CD4^+$ lymphocytes from T cell-sufficient *bg/bg nu/*$^+$ mice using an anti-CD4 monoclonal antibody increased their susceptibility to *Candida* infection of the tongue and esophagus (Cantorna and Balish, 1991), and confirmed that the $CD4^+$ cell population was crucial for protection of mice from orogastric candidiasis, although neither IL-2 nor IFN-γ was essential for an effective host response. In disseminated candidiasis, the different subsets of $CD4^+$ T helper cells, and cytokines produced by them, show a strong correlation with susceptibility and protection from infection (Romani, 1999), and parallels have been drawn between this and GI infection.

For example, BALB/c mice are resistant to systemic challenge with an avirulent isolate of *C. albicans*, and protective host responses are associated with a Th1 cytokine profile (Romani et al., 1993). In contrast, systemic infection of these mice with a virulent isolate causes early mortality, whereas GI colonization with the virulent yeast results in the production of both Th1- and Th2-type cytokines by $CD4^+$ cells from Peyer's patches and mesenteric lymph nodes, and clearance of the yeast from the intestine (Cenci et al., 1995). DBA/2Cr mice develop fatal disseminated candidiasis after intravenous infection with the avirulent strain of *C. albicans* (Romani et al., 1993), but intragastric inoculation with the virulent strain was again associated with the induction of Th1-type cell-mediated immune responses and eventual clearance of the infection (Bistoni et al., 1993).

These data suggest that activation of Th1-type cytokine responses is associated with recovery from orogastric *Candida* infection, but the nature of the effector pathways, and the relevance of the Th2-like response in the "resistant" BALB/c strain, have not yet been resolved.

4.2.2. Studies in Immunodeficient Mice

C. albicans is recognized as an organism that is uniquely adept in exploiting deficiencies in host responsiveness, and significant advances have been made in understanding yeast–host relationships by the use of mutant and genetically modified mice. In humans, cellular immunodeficiency, particularly that associated with HIV infection, substantially increases the risk of mucosal infections with *C. albicans*, and this condition has been modeled by the use of the T cell-deficient "nude" mouse.

Nude mice were shown to be markedly more susceptible to oral infection than euthymic controls (Farah et al., 2002b), and developed a chronic infection. There was extensive hyphal penetration of the oral epithelium, associated with infiltration of PMNLs and the formation of microabscesses. Mice of the CBA/CaH strain that are predisposed to severe tissue pathology (Ashman and Papadimitriou, 1987) acquired a greater fungal burden and developed more severe lesions after oral infection than the "low pathology" BALB/c mice. This is consistent with the regulation of tissue susceptibility by the *Carg1* gene (Ashman, 1998). Established infections in nude mice could be cleared by the adoptive transfer of syngeneic lymphocytes (Farah et al., 2002b), and the protective effect was mediated by the $CD4^+$, but not the $CD8^+$ lymphocyte subset. After reconstitution, IFN-γ and IL-12 were produced by lymphocytes from the cervical and submaxillary lymph nodes of the infected mice, but IL-4 and IL-10 were generally not detected.

Similar conclusions have been drawn from studies of orogastric candidiasis. Nude mice were unable to clear *Candida* from the stomach or the tongue (Balish et al., 1990), and did not develop either DTH or lymphoproliferative responses to *C. albicans* antigens. After intragastric or oral inoculation, both SCID mice, which lack functional T and B cells, and multiply-immunodeficient (*bg/bg*, *nu/nu*) mice, which are deficient in both T cells and phagocytic cells, develop a persistent GI infection, whereas mice that lack only phagocytic cells (*bg/bg*, *nu/*$^+$) are able to clear the infection efficiently (Cantorna and Balish, 1990). Transgenic epsilon 26 mice have defects in both natural killer cells and T cells and are highly susceptible to oro-esophageal and gastric candidiasis (Balish et al., 2001), but resistant to acute systemic candidiasis and systemic candidiasis of endogenous origin. Granulocytes were the major effector population responsible for protection against systemic infection (Balish et al., 2001), whereas T cells appeared to be essential for protection against mucosal infection. In contrast, B cell knockout (KO) mice, which lack both functional B cells and antibodies, were as resistant to orogastric candidiasis as immunocompetent controls (Narayanan et al., 1991), further substantiating a convincing case that cell-mediated immunity plays a central role in host protection against mucocutaneous candidiasis.

4.2.3. γ/δ T Cells

The γ/δ subset of T cells represents an important component of the mucosal immune system that has been implicated in the process of host defense against the yeast. In BALB/c and DBA/2 mice, oral infection with *C. albicans* resulted in an expansion of the γ/δ cell population (Chakir et al., 1994; Elahi et al., 2000), although the strains showed different kinetics, with the influx commencing on day 3 in the BALB/c mice, but on day 5 in the DBA/2 strain (Chakir et al., 1994). In both strains, the increase in γ/δ cell numbers was associated with a substantial decrease in the number of viable organisms recovered from the mucosal tissue. Oral colonization of B cell-deficient mice, which have a normal T cell response, increased the number of both α/β and γ/δ T cells in the GI mucosa (Jones-Carson et al.,

1997), and an accumulation of γ/δ T cells in the peritoneal cavity was also observed after intraperitoneal infection of immunocompetent and KO mice with *C. albicans* (Jones-Carson et al., 1995). In vitro, the γ/δ T cells enhanced nitric oxide production and macrophage candidacidal activity, and depletion in vivo abrogated expression of inducible NO synthase in the mucosa and enhanced susceptibility to *Candida* infection. Interestingly, mice lacking both α/β and γ/δ T cells were found to be susceptible to orogastric candidiasis, but not to acute systemic candidiasis (Jones-Carson et al., 2000), patterns of susceptibility that are similar to those seen in nude mice (Ashman et al., 2004).

4.2.4. Role of Cytokines

In systemic candidiasis, the roles of the Th1 and Th2 cytokines as regulators of host immune responses have been well established (Romani, 1999), whereas in oral infection, the identity of the cytokine mediator(s) of host resistance remains elusive and the relationship seems more complex. In general, IL-12 and IFN-γ were the dominant cytokines produced by lymphocytes from the draining lymph nodes of recovering animals (Farah et al., 2001a), but levels of IL-4 and IL-10 did not show any association with recovery from oral infection. TNF-α was the only cytokine that appeared to be unique to infected oral mucosa (Farah et al., 2002a). A comparison of host responses in the relatively resistant BALB/c or infection-prone DBA/2 mice showed that rapid clearance of *C. albicans* from the mucosa of BALB/c mice was associated with an early increase in levels of IL-4, IL-12, and IFN-γ in cells from the cervical lymph nodes, whereas in the infection-prone DBA/2 mice, expression of message for IL-4 was delayed, and the levels secreted were lower (Elahi et al., 2000). In BALB/c mice, monoclonal antibody neutralization of IL-4 increased the fungal burden and delayed the clearance of the yeast. Thus, IL-4 appears to be an important mediator of protection in oral candidiasis. In contrast, IL-12-deficient mice were highly susceptible to primary GI infection and showed an elevated production of IL-4 with a concomitant reduction in IFN-γ (Mencacci et al., 1998). Treatment of mice with GI candidiasis by administration of soluble IL-4 receptor (sIL-4R) accelerated clearance of the yeast from the stomach and stimulated Th1-associated resistance (Puccetti et al., 1994).

It might have been expected that the use of mice in which specific cytokine genes had been genetically deleted would have enabled some of these conflicts to be resolved; however, studies of candidiasis in cytokine KO mice has confused, rather than clarified the issue. In one study, IFN-γ KO mice showed increased susceptibility to both gastric and systemic candidiasis (Balish et al., 1998), whereas another reported no effect on either form of the disease (Qian and Cutler, 1997), and a third found increased mortality of the KO mice, although the increased susceptibility did not correlate with the extent of organ colonization (Kaposzta et al., 1998). Ablation of IL-10 increased resistance against both GI (Del Sero et al., 1999) and systemic (Vazquez-Torres et al., 1999) candidiasis, but deletion of the gene for IL-4 had no effect (Kaposzta et al., 1998). Mice lacking the homologue for the IL-8 receptor showed increased susceptibility to gastric and acute systemic candidiasis (Balish et al., 1999), with a slower influx of polymorphonuclear neutrophils into infected tissues, and a reduction in the candidacidal capacity of these cells.

In contrast, recent studies of oral candidiasis in IFN-γ, IL-4, IL-10, and iNOS KO mice have failed to demonstrate any alteration in the severity or course of the disease (Farah, unpublished data). In TNF-α KO mice, there was an early increase in the fungal burden in the oral cavity, but the duration of the infection was not different from controls. Infection in IL-12 KO mice,

however, was similar to that in T cell-deficient nude mice (Farah, unpublished data) in that the fungal burden in the oral cavity increased and the infection became chronic, persisting undiminished for at least 3 months. The precise role of IL-12 as a mediator of host resistance, the anomalous position of IFN-γ, and the importance of IL-4 remain to be resolved. Our failure to identify a particular cytokine or set of cytokines as a crucial link in the effector pathway against the yeast may be due to a redundancy among the cytokines that masks the effect of gene deletion in KO mice; however, it is difficult to evaluate the significance of such functional overlap in vivo. Alternatively, there may exist additional effector pathways; or there may be significant, as yet unidentified, differences between the experimental models. Based on the work undertaken by the authors, a hypothetical pathway for activation of effector cells in oral candidiasis is presented in Fig. 4.2.

4.2.5. Candidacidal Effector Mechanisms

Although T cells have been shown to be essential for recovery from oral candidiasis, neither αβ nor γδ T cells have any candidacidal or candidastatic effect per se. Therefore, other cell types are required for the eradication of the yeasts from the infected mucosa.

Depletion of neutrophils or inactivation of macrophages/monocytes increased the severity of oral infection in BALB/c mice, but had a lesser effect in the CBA/CaH strain (Farah et al., 2001a). Ablation of both cell populations further increased infection in BALB/c mice, but dramatically exacerbated the fungal burden in the CBA/CaH strain. In the absence of activation, *Candida* killing by phagocytic cells (both neutrophils and macrophages) is relatively inefficient, but the candidacidal potential of both cell types is significantly enhanced by exposure to Th1-type cytokines, such as IFN-γ and TNF-α. It thus seems that phagocytic cells play a more dominant role in the susceptible CBA/CaH mice than in the resistant strains, although there was no significant difference in clearance of chronic infections in nude mice of either strain after reconstitution with lymphocytes (Farah et al., 2002b).

Nevertheless, recent studies in nude mice have thrown further light on the critical role of the bone marrow in the determination of tissue susceptibility to infection with *C. albicans* (Ashman and Papadimitriou, 1992). Bone marrow colony formation in vitro was unchanged in immunocompetent mice infected either orally or systemically (Wanasaengsakul and Ashman, 2004); however, it was significantly depressed in chronically infected, "high pathology" CBA/CaH nude mice, compared to BALB/c. Reconstitution of nude mice with T cells significantly increased the colony-forming response in infected BALB/c, but not in infected CBA/CaH mice. These results suggest that T cell-mediated enhancement of phagocytic cell production by the bone marrow may be one effector pathway.

The importance of phagocytic cells in host resistance was confirmed by treatment of SCID mice with cyclophosphamide (Balish et al., 1993). This caused severe neutropenia and impairment of innate immune mechanisms, and enhanced their susceptibility to mucosal candidiasis. Furthermore, impairment of macrophage function in SCID mice by administration of poly(I–C) increased susceptibility to disseminated candidiasis of endogenous (GI tract) origin (Jensen et al., 1992, 1994), but the resistance of immunocompetent controls to mucosal candidiasis was not altered by treatment with poly(I–C) alone. Interference with both macrophage and neutrophil function was necessary to render these mice susceptible to the disease (Jensen et al., 1993).

GI colonization of athymic (Jones-Carson et al., 1995) and SCID (Vazquez-Torres et al., 1995b) mice with *C. albicans*

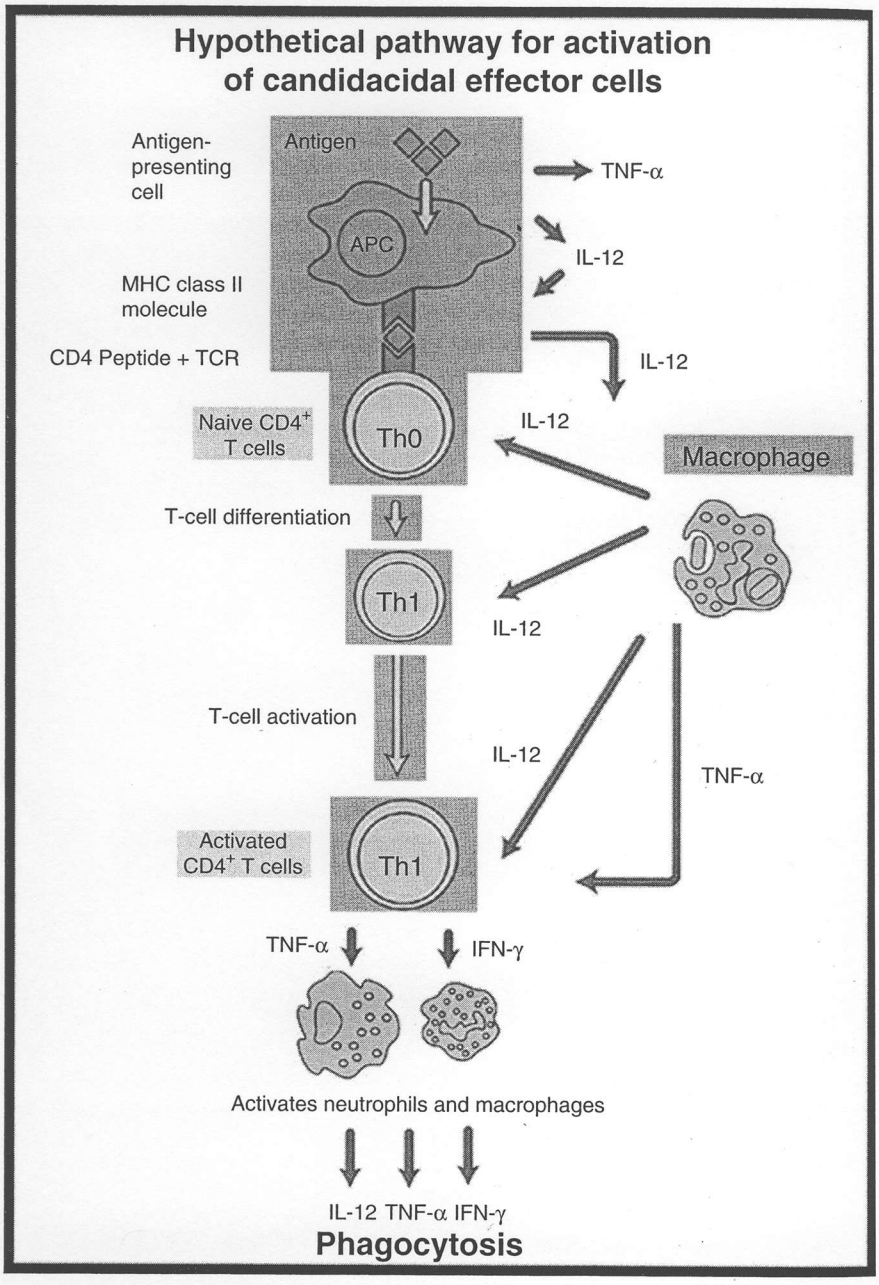

Figure 4.2. Hypothetical pathway for activation of effector cells in oral candidiasis. Stimulation antigen-presenting cells (APC) by antigen(s) of *Candida* results in the production of TNF-α, wh increases recruitment of inflammatory cells, and augments the candidacidal activity of phagocytic c early in the course of infection. The APCs secrete IL-12, which drives the differentiation of naïve CI T cells, and induces a Th1-type cellular immune response. The Th1 cytokines (IFN-γ and TNF-α) both separately and synergistically to promote *Candida* killing by both neutrophils and macropha while both IL-12 and IFN-γ act on the APC in a feedback loop to enhance their activation.

stimulated the expression of inducible nitric oxide synthase (iNOS) in the gastric and oral mucosa. Inhibition of NO production in SCID mice enhanced their susceptibility to orogastric candidiasis, but in athymic mice, the expression of iNOS was controlled by γ/δ T cells (Jones-Carson et al., 1995) because depletion of these cells not only abrogated the expression of iNOS in the tongue and stomach, but also increased susceptibility to orogastric candidiasis. In contrast, immunocompetent mice did not express iNOS in the orogastric mucosa after they were monoassociated with *C. albicans* and mucosal candidiasis was not exacerbated after treatment with NOS inhibitors (Jones-Carson et al., 1995). However, no direct correlation was demonstrable between nitric oxide production and *Candida* killing (Vazquez-Torres et al., 1995a), suggesting that nitric oxide was candidastatic rather than candidacidal, and was associated with or induced other macrophage candidacidal mechanisms.

In inbred mice, NO was detected in the effector phase of the response against oral infection (Elahi et al., 2001b). Concentrations of NO in the saliva of mice increased after infection and saliva from infected mice inhibited the growth of yeast in vitro. Neutralization of IL-4 in vivo caused a marked reduction of NO levels in saliva (Elahi et al., 2001b), and in cultures of cervical lymph node cells after stimulation with *C. albicans* antigen. Conversely, treatment with NG-monomethyl-L-arginine (MMLA), which inhibits NO synthesis, led to an increase in *C. albicans* in the oral cavity and a concomitant abrogation of expression mRNA for IL-4, but not IFN-γ, in lymphocytes from the draining lymph nodes. Paradoxically, inhibition of IL-4 production was accompanied by an increase in IFN-γ production in susceptible DBA/2 mice (Elahi et al., 2001b), which tends to argue against a strict Th1/Th2 dichotomy as a determinant of resistance and susceptibility in oral candidiasis. However, in a different model, treatment of orally infected mice with aminoguanidine (AG), which inhibits iNOS, had no effect on the magnitude or duration of infection (Farah, unpublished data).

4.2.6. Role of the Infectious Challenge

The above review of results from models of oral and GI candidiasis has identified a number of common themes in mechanisms of host defense, but anomalies still remain. The contrast between the results of Elahi and Farah is of particular interest, as both groups were using the same isolate of the yeast (3630, from the Mycology Reference Laboratory at the Royal North Shore Hospital). After oral infection, DBA/2 mice showed a bimodal pattern of colonization (Chakir et al., 1994), a feature reproduced by Elahi et al. (2000), whereas in the experiments of Farah (Ashman et al., 2003), only a single peak was observed. It seemed probable that the course of oral infection in this mouse strain was influenced by the actual technique used for infection. Elahi et al. (2000) pressed the yeast onto the gums using a small swab, whereas Farah et al. (2001a) introduced a suspension of yeasts into the mouth, without damaging or traumatizing the oral mucosa. Thus, in the former case, microtrauma may have facilitated both deeper penetration of the yeast and the rapid elicitation of protective cell-mediated immune response. When inoculation was atraumatic, adhesion of the yeast to the oral mucosa may have been more difficult to establish, and innate immune mechanisms more directly involved, leading to more effective resistance against initial colonization by the yeasts.

This observation led us to test the hypothesis that there was a gradation in host responses from innate to adaptive immunity, which was determined by the severity of the infectious challenge. Nude mice develop chronic infections after oral challenge with 10^8 yeasts. However, as the doses used for infection were progressively decreased, the magnitude of the fungal burden in the

chronically infected mice decreased proportionally, until eventually, the nude mice were able to clear the infection completely (Ashman et al., 2004). Thus, the requirement for $CD4^+$ T cells for clearance of the infection in these mice apparently relates to the immunological deficit that permitted the infection to become established—a situation comparable to the susceptibility to oropharyngeal candidiasis of HIV/AIDS patients. It is noteworthy that in neither the human nor the experimental model is there any systemic dissemination of the mucosal infection, suggesting that innate immunity may also play a major role in the response against systemic disease.

4.2.7. Experimental Immunity and Protection

The genetic background of the mice influences the nature and magnitude of *Candida*-specific memory responses in systemic infection, in that the susceptible CBA/CaH mice tend to show lower cell-mediated immune responses (Ashman, 1990), but greater levels of antibody-mediated protection (Ashman and Papadimitriou, 1988) than the more resistant BALB/c strain. However, following oral infection, *Candida*-specific DTH responses developed in the relatively infection-prone DBA/2, but not in the more resistant BALB/c mice (Chakir et al., 1994). Humoral immune responses were detectable after infection had resolved (Elahi et al., 2000), but levels of serum IgG and salivary IgA antibodies were higher in BALB/c than in DBA/2 mice. In neonatal mice, primary infection of the GI tract primed them for enhanced DTH responses and conferred protection from systemic challenge as adults (Domer, 1988), whereas adult mice infected via the oral cavity, but not intravenously, were protected against a second oral infection (Farah, unpublished data). However, neither serum nor cells from orally or systemically immunized mice, transferred into naive recipients, was able to protect against oral challenge.

5. Prospects for Vaccination

Vaccination against *C. albicans* is a challenge, because of the commensal nature of the organism and because humans are colonized with the yeast in the early postnatal period. A thorough understanding of the mechanisms that confer protective immunity is fundamental to successful vaccine design, particularly in view of the problems inherent in delivering protection to mucosal surfaces. Direct immunization of the oral cavity has been shown to have therapeutic potential in mice (Elahi et al., 2001a). Mice given the oral vaccine had a reduction in colonization associated with increased levels of secretion of IFN-γ and IL-4 from the regional node cells and increased levels of nitric oxide in saliva. The use of mannan–protein conjugates as immunogens has been shown to have potential (Han et al., 1999), but perhaps a more exciting approach is the use of cytokines as adjuvants (Deepe, 1997). Another approach to vaccination against oral *C. albicans* infection has been the use of oral *Lactobacillus acidophilus* (LAVRI-A1) to induce local cytokines and enhance clearance (Elahi et al., 2003). Protective antibodies against *C. albicans*, including a human recombinant mAb specific for *C. albicans* hsp90 (Mycofab), are also being evaluated (Matthews and Burnie, 2001).

Vaccination of the immunocompromised host represents a further challenge, as vaccines that rely on a competent immune system are unlikely to be useful in immunodeficient patients. In these cases, protective antibodies could be more beneficial than cellular immunity because of the longevity of the circulating antibodies. Ultimately though, a vaccine in these patients will not succeed unless it is linked with attempts to restore the integrity of the immune system. This might be done by delivering the vaccine with cytokines that are known to enhance the immune response

(Deepe, 1997), or introducing the vaccine with immunocompetent T or B cells, to promote its immunogenic potential.

6. Conclusion

There is now convincing evidence, both clinical and experimental, that cell-mediated immunity is crucial to host recovery from oral or OPC. This infection, however, appears to demonstrate a gradation between the involvement of the innate and adaptive immune responses, depending on the severity of the challenge. $CD4^+$ lymphocytes are certainly involved in the more severe manifestations of the disease, but the precise pathways through which they interact with the phagocytic effector cells that clear the infection have yet to be completely elucidated. Oral immunization can confer protection, but again, the mechanisms by which this is achieved require further investigation.

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