Review

Candida Infections of Skin : Potentiation, Protection and Prevention

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In recent years candida species have emerged as a major human fungal pathogen, causing both superficial mucocutaneous and life-threatening systemic disease in alarming proportions around the globe. Fungal infections of skin generally reflect some breakdown in the host's defense system, which, as we know, is quite complex. In the infectious process, however, both host and parasite factors must determine the outcome of any mutual encounter. This review highlights the multitude of host and fungal factors which potentiate skin infections with candida species (1). In addition, I will consider natural protective factors which might be amplified to treat or prevent such infections in susceptible individuals. The bibliography is limited to more inclusive reviews and specific points for emphasis.

I. Host factors that influence candida infection

A. Local conditions

Normal bacterial flora of the skin tends to be protective against candida colonization on the skin (2). Conditions that upset the flora, such as constant moisture and/or occlusion, promote candida growth and contribute to a skin condition called *erosio interdigitale*. Topical antifungal drugs are usually ineffective unless the skin is kept dry. Occlusion with, for example, a diaper, makes the situation worse. Trauma and moisture also are believed to be main predisposing factors that allow candidiasis to evolve. In addition, chronic use of topical corticosteroids changes skin flora

and other protective forces, allowing infection to proceed.

B. Systemic conditions

Immunodeficiency : Cell mediated immunity has been considered to be primarily involved in host defense mechanisms, despite the fact that circulating antibody and complement are known to kill candida organisms. Consequently, patients with acquired immunodeficiency syndrome (AIDS) and/or infected with the human immunodeficiency virus (HIV) are particularly susceptible to candidiasis (3). Skin and oral lesions often show extensive involvement and in many cases the lesions extend from the tongue and buccal mucosa down the esophagus. In severe cases a rupture of the esophagus has been reported in AIDS patients.

Hematopoietic Cell System : Polymorphonuclear leukocytes (PMNs), and to a lesser extent platelets, are cell sources that have a non-immune defense function against candida infection. PMNs have both oxygen dependent

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and oxygen independent systems (myeloperoxidase and defensins), that specifically kill candida organisms (4-7). Mucocutaneous disease is seen in patients with leukopenia and granulocytic leukemia. The function of platelets may be due to the content of β lysin, an antimicrobial, low molecular weight histidine-rich protein (7).

Endocrine Disturbances : A variety of abnormalities and dysfunctions in endocrine glands predispose individuals to candidiasis. Pregnancy and polyendocrine abnormalities are examples. As many as 40 percent of patients with polyendocrine abnormalities associated with mucocutaneous candidiasis have died of adrenal failure (8). Diabetes is another condition that develops extensive infection on the tongue and buccal mucosa.

Tissue Factors and Secretions : Humans and animals have numerous bodily secretions which demonstrate candidacidal activity. Among the more than forty identifiable proteins and peptides obtainable from salivary glands, the family of histatins (a novel family of histidine-rich proteins) especially exhibit this ability to kill the pathogenic yeast. At least seven of these proteins have been isolated and cloned (9, 10). Breast milk contains lactoferrin of the transferrin superfamily, which also may regulate microbial growth (7). A unique high molecular protein which contains about 7 percent histidine-residues was purified by Kashima and others from rat epidermis (11). This protein has been shown to inhibit growth of *Candida albicans*, but not *Candida parapsilosis*.

Systemic treatments can predispose to candidiasis. Oral therapy with tetracycline classically provides candida organisms with a growth advantage by reducing the numbers of bacteria on the mucous membranes and in the gut (1). Aggressive neoplastic treatment of cancers is a well known predisposing factor, but susceptibility seems to be due to complex effects of the drugs on both the host and the organism (12). Thus some anticancer drugs actually decrease fungal adherence at the same time that they reduce immune defenses. By a

similar intricate formulation, systemic corticosteroids seem to have potentiating rather than inhibitory effects.

Nutrition is another systemic factor. Zinc deficiency clearly predisposes to mucocutaneous candidiasis both experimentally and in some patients (13). Candidiasis is often seen in patients with acrodermatitis enteropathica and the fungus infection heals with zinc therapy. Carbohydrate excess and vitamin deficiencies also are reputed to be predisposing factors (1).

A number of miscellaneous systemic factors can contribute to fungal susceptibility. Immaturity and slow development undoubtedly account for some cases of recalcitrant candida infection in infants and young children. Debility of aging and advanced cancer play a role, and prosthetic surgery, particularly heart valve and joint replacements, have accounted for candidal infections in a significant number of patients (1).

II. Parasite Factors

To cause disease, the yeast must first attach to the skin surface, grow and invade the tissues to produce inflammation and morbidity. Attachment is a crucial step in the sequence and the ability to attach depends upon a variety of factors. As mentioned earlier, bacteria on the skin surface tend to prevent attachment. The host cell type is important, but our focus is on the yeast. Species and strain differences, phase of growth, and, up to a point, the concentration of the organisms on skin all have significance (14). *Candida albicans*, the major fungal pathogen of man, always shows the greatest degree of adherence in comparative attachment studies, no matter what assay procedure is used. *C. tropicalis*, a known pathogen, tends to come in second. But some of the other species such as *C. pseudotropicalis*, *C. parapsilosis* and the emerging pathogen *C. krusei*, all show much less tendency to adhere to cells in both in vitro and in vivo assays (14, 15). Also the phase is important. During the budding phase candida organisms are more

likely to attach than during the hyphal phase. Other factors influencing attachment include drugs in the environment. Fluorinated corticosteroids in an in vitro test enhanced adhesion; while hydrocortisone, a variety of antifungal drugs and numerous antineoplastic drugs all tended to decrease adhesion in a model where *C. albicans* adherence was measured in vitro (16, 17).

The mechanisms of yeast adhesion can be specific, with the fungus binding through adhesion-receptor complexes on different cells, or they can be nonspecific, related to the hydrophobicity or ion bonding between the cell surface and the yeast. Also, aggregation and coadherence will influence binding to cell surfaces both in vivo and in vitro (14, 15). However, these factors, especially hydrophobicity are not the long-sought virulence factors (18).

While much is known about the structures and molecules on the surface of bacteria which account for attachment to cells, somewhat less is known for certain about candida. Nonetheless, the majority of studies have reported that yeast cell surface polysaccharides or mannoproteins, which constitute more than twenty percent of the yeast cell wall, are the major molecules responsible for adhesion to mammalian cells. Chitin, another component of the yeast cell wall, is an unlikely candidate for this activity but has not been entirely ruled out yet (14, 19). By detailed study, the yeast cell wall can be divided into between 5 and 8 distinct layers (14, 20). The outermost layer of the yeast cell wall is composed ultrastructurally of a thin and not necessarily continuous accumulation of microfibrillae, and this is one of the areas where mannoproteins have been identified. It has even been suggested that the fibrillar layer is the equivalent of the fimbriae of bacteria, structures known to be responsible for cell adhesion (14).

Above the plasma membrane of the yeast, β glycan/chitin and pure β glycan form distinct layers. Mannoproteins appear somewhat more superficial and enzymes are embedded both within the mannoprotein and glycan

sublayers. In the budding form of candida, the amount of chitin extracted from the cell wall amounts to about 1 percent or less and is concentrated and crystallized at budding points (20). Nevertheless, it is not totally correct to assign each of these layers to a single sugar or polysaccharide protein complex, because they are not so much segregated as enriched in these molecules at the different layers (20). Furthermore, it is known that chitin spontaneously tends to form microfibrils, so that just as in bacteria, where there are several cell binding structures and molecules (21), it may likely turn out that in candida the situation concerning cell adhesion is more complex than currently conceived.

From a medical standpoint, the most important question to ask is how to determine the virulence of candida species, subspecies and strains. Several typing schemes have been devised for such differentiation, including DNA fingerprinting, but to date, the majority have not been able to consistently distinguish candida of high and low virulence (22, 23). The four characteristics which seem most important in determining virulence are: 1) adherence, which we discussed; 2) ability to form true hyphae in addition to mycelial formation; 3) secretion of proteinases; and 4) resistance to phagocytosis (22). Once the yeast has attached and colonized the skin, if it is to become virulent, it must invade. This is generally believed to occur through secretion of extracellular acid proteinases, one of which appears to be an aspartic proteinase (24). The atomic model of a crystallized aspartic proteinase of the HIV-1 virus has been published (25) and when this knowledge becomes available for *Candida albicans*, it may be possible to develop topical analogs to interdict the virulence of this yeast. All four of the virulence characteristics are most highly developed in *Candida albicans* and to lesser degrees in other of the candida species (22).

III. Defense Strategies

The final point for discussion deals with

defense strategies that might protect patients from candida infections. The most direct approach would be to develop more broad spectrum antibiotics which also kill *C. albicans* and/or to develop more potent and specific antifungal drugs against the yeast. A whole industry is geared to this endeavor.

Another approach would be to replace host defects, and here the most important broad group to consider might be use of immunomodulators which could induce specific immunologic responses, as was done some years ago, using transfer factor in mucocutaneous candidiasis (26). However, non-specific immuno modulators are more widely used in medicine today. Thus, experimental contact sensitization with DNCB or other chemicals has been used in such diverse diseases as melanoma, warts, alopecia areata, and AIDS with measurable improvement in certain cases. Replacement of leukopenic patients with granulocyte colony stimulating factor is another possibility. In specific instances hormones, secretions, or nutrients might be replaced. Thus patients on intravenous alimentation in the past who developed acquired acrodermatitis enteropathica due to zinc deficiency were rapidly healed when zinc was added to the iv fluids.

Yet another approach might be to take advantage of the myriad of the non-immunologic defenses that are becoming available. Thus, Magainins are antimicrobial peptides from frog skin (27) which are being processed for possible use in man, and people have considered using Defensins from the polymorphonuclear leukocytes (5). The same might be conceivable for the histidine rich protein isolated from skin (11). In addition, the large family of histatins isolated from the parotid and submandibular glands, which have powerful antimicrobial activity, might also be designed for human use because the cDNA's are known (9, 10).

Still another idea would be to interfere with microbial adherence. For example, it has been shown that suboptimal dosing with antibiotics while not killing bacteria, will prevent them

from adhering to mammalian cells (28, 29) and can be utilized as a strategy in preventing bacterial infections. The same concept has been detected in in vitro testing with antifungal agents against the adherence of candida species (17).

Lectinophagocytosis is a molecular mechanism whereby recognition between host cell surface glycoconjugates and bacterial lectins enhances the phagocytosis of microorganisms (30). We also know that glycoconjugates coat the surface of all epidermal cells and these polysaccharide coatings become ever more complex as the cells terminally differentiate (31, 32). When skin is wounded, this pattern is disrupted temporarily at the very time when the potential for infection increases (33). I suggest that treatment of wounded skin or cornea with appropriate glycoconjugates may deter infection and actually speed healing. Finally, and highly speculative, would be to construct candida clones which lack virulence but compete for attachment. How might that work? It has been known for some time that the *C. albicans* genome is diploid with 15-20 femptograms of DNA per haploid (34). Fortunately a great deal has been learned about the molecular biology of *Saccharomyces cerevisiae* and recent information seems to indicate that *C. albicans* genome will be very much like *Saccharomyces* (34), so that few surprises are expected. Furthermore, there are 6 chromosomes and, more than 13 genes have already been cloned (34); and most exciting the two crucial pieces of information needed before anyone could move ahead with manipulation of the *C. albicans* genome have been discovered, namely that: 1) integrative transformation is possible, a mechanism well studied in *Saccharomyces cerevisiae* (34). Under this scheme, when a candida organism is transformed with an integrating vector, such as pMK3, the plasmid inserts into the genome by homologous recombination producing repeats of a candida sequence such as ADE2 separated by a vector sequence (35); and 2) gene disruption has been demonstrated in *C. albicans*. It turns out that

free-DNA termini of *C. albicans* are recombinogenic and in one study the cloned ADE2 gene was inserted into the middle of a candida URA3 gene and the linear fragment carrying this construct was then used to transform candida (36). As more information is gained about the *C. albicans* genome many other types of constructs are possible (37) and the original goal to take away virulence may actually be attainable.

The above analysis does not imply that the problem of epidemic candidiasis, a world-wide affliction is imminently under control, but this admittedly brief and limited review has emphasized that humans possess a multitude of natural defenses against candida infection; and it should be possible to amplify some or all of them to not only protect susceptible individuals, but to develop preventative therapies for all populations.

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