Society for Pediatric Research Presidential Address 1994: Yeast as Metaphor

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About 7 years ago, I was asked to evaluate an 8-month-old boy who had been devastated by pneumococcal meningitis. On intravenous hyperalimentation and Decadron for irreversible brain edema, he now had positive blood and urine cultures for *Candida albicans*. And had I been able to peer into that baby's bloodstream, I would have viewed an epic battle between yeast and neutrophil: on the one side, a primitive eukaryotic cell, on the other, a sophisticated killing machine (Fig. 1).

Such a story is all too common today, with *C. albicans* the leading cause of fungal infection in our hospitals. But why *Candida*? Why not some other yeast, like *Saccharomyces cerevisiae*? Both of these species are indigenous to the human gastrointestinal tract, and many of the genes encoding structural proteins, such as tubulin or actin, are identical. And yet *S. cerevisiae* is invariably kind to us, whereas *C. albicans* causes more than 60% of all fungal infections, with a fatality rate that approaches 30%, even with appropriate antifungal therapy.

In the quest for answers, there were some superficial starting points. For example, like my patient, the host who develops candidal fungemia typically evidences one or more risk factors. Antibiotic use, hyperglycemia, steroids, indwelling catheters, and neutropenia—each may be invoked when neonates, diabetics, intensive care unit patients, or neutropenic hosts develop invasive candidal infection, and yet none of these, singly or together, explains the disease.

Then we were greatly helped in our search by two observations: First, Heidenreich and Dierich (1) reported that sheep erythrocytes bearing the C3 fragment iC3b rosetted with pseudohyphae from *C. albicans*. Subsequently, Edwards (2) demonstrated that a murine monoclonal antibody, recognizing the type 3 human complement receptor (CR3), bound to *C. albicans* pseudohyphae. These observations therefore suggested that *C. albicans* bore specialized surface proteins that mimicked the complement receptors on human leukocytes, CR3 and its closely related homolog CR4, members of the β_2 subset of the integrin superfamily. Integrins direct adhesion and cell-to-cell communication on a host of eukary-

otic cells (Fig. 2) (3) and are thought to have evolved from a primordial gene, as yet undiscovered, but it was a great leap for these investigators, across more than one billion years of evolution, to surmise that a primitive and unsophisticated little yeast might hold the key to an evolutionary mystery.

I will briefly describe the evidence for the classification of these candidal complement receptors as integrin analogs and for the primacy of their role as adhesins in the pathogenesis of candidal infection.

The mammalian integrins, a family of 16 α -subunits and eight β -subunits, are expressed as heterodimeric transmembrane proteins on a wide variety of mammalian cells. In present taxonomy, integrins are grouped in subsets on the basis of their β -subunits. As shown in Figure 2, two of the β_2 integrins share antigenic, structural, and functional homologies with candidal surface proteins: α_M , which is also known as Mac-1, CD11b, or CR3, and α_X , which is known as p150,95, CD11c, or CR4. α_M and α_X are polypeptides of 165 and 150 kD, respectively; in leukocytes, they are expressed in association with a common β -subunit of 95 kD.

There are several functional domains on mammalian integrins that deserve special mention. Both the α - and β -subunits have distinct cytosolic tails that mediate intracellular signaling via interactions with actin and talin, and both have highly conserved transmembrane domains (3). In the extracellular region, α_M and α_X exhibit an inserted domain, or I-domain, that binds the C3 ligand iC3b and is recognized by the monoclonal antibody Mn41. In addition, there are three calcium-binding sites that are required for ligand binding. Lastly, the carboxy terminus of the extracellular domain is recognized by a second monoclonal antibody, called OKM1.

It is presently thought that the extracellular domains of both the α - and β -subunits cooperate in the recognition and binding of integrin ligands in the extracellular matrix; many but not all of these ligands are distinguished by the tripeptide sequence of arginine, glycine, aspartic acid, or RGD.

Graphic evidence of antigenic relationship between the mammalian β_2 integrins and integrin analogs in yeast is this immunofluorescent micrograph from our laboratory, in which incubation of yeast-phase *C. albicans* with anti- α_M monoclonal antibodies such as anti-Mo1-94 results

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Figure 1. Scanning electron micrograph of *C. albicans* pseudohyphae and human neutrophils ($\times 5000$) (reprinted from Gower DJ, Crone K, Alexander E, Kelly DC 1986 *Candida albicans* shunt infection: report of two cases. *Neurosurgery* 19:111–113, with the permission of the publisher).

in circumferential immunofluorescence (Fig. 3, panel B), whereas incubation with isotype controls yields no fluorescence (Fig. 3, panel D).

In our laboratory, we have used flow cytometry with saturating concentrations of anti- α_M monoclonal antibodies to quantitate expression of the integrin analog among several species of yeast (Table 1). For any given *C. albicans* isolate, nearly 80% of the population binds a variety of integrin monoclonal antibodies, whereas expression is considerably reduced in other *Candida* species and virtually absent in *S. cerevisiae*. This hierarchy of fluorescence accords quite closely with the frequency of isolation of *C. albicans* and other yeast species from immunocompromised hosts (Table 1): *C. albicans* is most frequent, followed by other *Candida* species and, lastly, by *S. cerevisiae*.

In addition, conditions of clinical relevance such as hyperglycemia, use of steroids, or temperature may also influence integrin expression. Growth of yeast cells in medium containing ≥ 20 mM D-glucose, growth in the presence of 10^{-9} M hydrocortisone, or growth at 37°C —all increase expression of the integrin analog in *C. albicans* by as much as 2- to 6-fold (4, 5). These conditions suggest not only possible clinical correlates but potential regulators of integrin gene expression as well.

Structural similarities are no less intriguing. You will recall that α_M and α_X had molecular masses of 165 and 150 kD, respectively. Isolation of the integrin analog from a cytosolic extract of *C. albicans* yields a single band of 165 ± 15 kD under nonreducing conditions.

Functional analogies between the leukocyte integrins and the integrin analog in *C. albicans* include the binding of the ligand iC3b with identical affinity constants (4). This suggests that both candidal and mammalian inte-

Figure 2. Model of functional domains of the β_2 integrins in mammalian cells (modified from Albelda SM, Buck CA 1990 Integrins and other cell adhesion molecules. *FASEB J* 4:2868–2880).

grins contain an I-domain. Moreover, the conceptual model (Fig. 2) indicates that integrins in mammalian cells mediate adhesion to other eukaryotic surfaces, typically by recognition of RGD sequences in matrix proteins.

Similarly, in *C. albicans*, adhesion to the epithelium of the gastrointestinal tract stands as a first step in the pathogenesis of candidal infection. Having colonized the host's mucosal surfaces, *C. albicans* and some related species may then invade beneath the mucosal barrier, enter the vascular space, and replicate with impunity to cause hematogenously disseminated disease. We therefore asked whether epithelial adhesion of *C. albicans* could

Figure 3. Phase-contrast (A, C) and immunofluorescence microscopy (B, D) of a blood isolate of *C. albicans* after incubation with anti-Mo1-94 (B) or the isotype control MsIgM (D) (micrograph is reprinted from Gustafson KS, Vercellotti GM, Bendel CM, Hostetter MK 1991 Molecular mimicry in *Candida albicans*: role of an integrin analog in adhesion of the yeast to human endothelium. *J Clin Invest* 87:1896–1902, with permission of the publisher).

involve RGD sequences and, if so, whether we could use these strategies to block adhesion and possibly to avert the subsequent complications of invasive infection.

Using cervical epithelial cells, Dr. Catherine Bendel demonstrated that the degree of epithelial adhesion in *Candida* species reproduced quite closely the pattern of integrin expression quantitated by flow cytometry: epithelial adhesion was greatest for *C. albicans*, reduced for other *Candida* species, and virtually absent in *S. cerevisiae* (Table 1).

However, the contention that the integrin analog mediates epithelial adhesion requires more explicit evidence, both at the level of the yeast and from the standpoint of the epithelial cell. By immunofluorescence and immunoprecipitation, Dr. Eric Michael and Dr. Bendel (7) demonstrated that epithelial cells secrete at least two potential ligands for yeast adhesion: iC3b and fibronectin.

In iC3b, the RGD tripeptide is localized to the carboxy terminus of the α' chain; in contrast, the C3d subdomain is more centrally located within the α' chain and can be excised from the iC3b molecule but does not contain an

RGD sequence. Similarly, among several binding domains for heparin, for collagen, and for fibrin, fibronectin contains an RGD sequence in its cell-binding domain. The amino acid sequences flanking the RGD peptide are quite different in fibronectin and iC3b.

In experiments to block candidal adhesion, preincubation of *C. albicans* with 10- μ M concentrations of bovine serum albumin, fibronectin, or C3d had no effect on adhesion. In contrast, purified iC3b inhibited epithelial adhesion of *C. albicans* by 80% (Fig. 4). Additional experiments pointed to the importance of the RGD tripeptide in iC3b and its specific flanking sequence in mediating this interaction (Fig. 5). RGD peptides from iC3b that were less than nine amino acids in length, or a series of RGD peptides from fibronectin (seven to 23 amino acids) failed to block adhesion of *C. albicans* to epithelial cells. An RGK peptide was similarly ineffective. In contrast, RGD peptides of nine, 10, and 15 amino acids successfully blocked the adhesion of *C. albicans* to human epithelium, provided that they contained the correct iC3b flanking sequence (7).

This slide (Fig. 6) is affectionately called “the tap-dancing yeasts,” and in my book, *C. albicans* is the Fred Astaire of fungi. As *C. albicans* colonizes the oropharynx or the lumen of the gastrointestinal tract, its complement receptor or integrin analog contacts iC3b molecules studing the surface of the epithelial cell. Like the mammalian integrins, the integrin analog in *C. albicans* recognizes the RGD site and specific flanking residues in iC3b, and adhesion occurs. By flow cytometry, protein, and genetic studies, species such as *C. parapsilosis*, *C. glabrata*, *C. krusei*, or *S. cerevisiae* fail to express an integrin analog and therefore lack the “adhesion advantage” that it confers.

This adhesive interaction can be interrupted from the epithelial side with antibodies to iC3b, the secreted ligand, and from the candidal side with monoclonal antibodies against human α_M and α_X , with purified iC3b or, excitingly, with our new iC3b peptides (7). The door

Table 1.

Yeast species	Percentage of isolates from immunocompromised hosts*	Percentage fluorescing with OKM1†	Percentage of adhesion to epithelium‡
<i>C. albicans</i>	68.5	73.4 $\pm$ 3.6	44.0 $\pm$ 1.9
<i>C. tropicalis</i>	12.9	23.2 $\pm$ 6.6	38.8 $\pm$ 3.6
<i>C. glabrata</i>	10.7	27.2 $\pm$ 2.5	12.0 $\pm$ 6.5
<i>C. parapsilosis</i>	3.7	6.1 $\pm$ 1.9	19.3 $\pm$ 6.9
<i>C. krusei</i>	1.5	0.4 $\pm$ 0.2	20.9 $\pm$ 3.5
<i>S. cerevisiae</i>	0.8	3.4 $\pm$ 1.5	9.9 $\pm$ 3.6

* Data from Ref. 6.

† Data from Ref. 8.

‡ Data from Ref. 8.

Figure 4. Blockade of *C. albicans* adhesion to human epithelium with purified proteins (data taken from Ref. 7).

Figure 5. Blockade of *C. albicans* adhesion to human epithelium with RGD peptides (data taken from Ref. 7).

therefore opens to the possibility that we may be able to protect susceptible patients from candidal infection, or at least reduce their risk, by using these peptides to inhibit colonization.

Recent work from Greg DeMuri, Cheryl Gale, Mark McClellan, and Marilyn Meinke in my laboratory has led to the identification of several candidate integrin genes for *C. albicans* and related species. This model, once hypothesis but now fast becoming fact, should help to explain our present understanding of their relationships (Fig. 7).

In *Saccharomyces cerevisiae*, a nonadherent yeast, a gene encoding an intracellular myosin-like protein found in the cytoplasm surprisingly contains a sequence resembling an integrin I-domain. A fusion protein containing this portion of the gene product binds the I-domain ligand iC3b and is recognized by Mn41, the monoclonal antibody specific for this epitope. OKM1 and other integrin monoclonals do not bind. Lack of a signal sequence and the absence of a hydrophobic transmembrane domain

Figure 6. Interaction between the integrin analog and epithelially synthesized iC3b in the adhesion of *C. albicans* to human epithelium.

relegate this protein to the cytosol in *S. cerevisiae*, where it functions in intracellular protein transport (9). Thus, failure of surface expression predicts failure of adhesion.

In *C. albicans*, in contrast, a 4.9-kb open reading frame encoding a putative α -subunit integrin analog has been isolated. The polypeptide structure of the gene product shows several possible areas of homology: an I-domain, a transmembrane region, and interestingly, its own RGD site for yeast aggregation. Thus, at least one candidal gene with α -integrin homologies has been identified.

Analogues of the mammalian β -subunit have been identified in both *C. albicans* and *C. tropicalis*, the second-most adherent candidal species (10). So we are now poised to understand the genes that encode a panoply of integrin-like proteins and, in the case of glucose and steroids, the promoters and regulatory elements that control them, as we move from primitive eukaryote to its more virulent cousin *Candida* and on to the mammalian cell.

The traditional stopping point, of course, is here, but to halt now would be to emphasize the facts at the expense of the metaphor. And that, as a former English major, I am forbidden to do, because we learn much from science apart from fact. What we learn biologically is that a simple yeast, a primitive eukaryote, carries genes related to a much more complex system in humans, reaching across eons of evolution to establish those commonalities of biologic conservation. What we learn philosophically is that we can take a worthy lesson from that yeast, and we can apply that lesson to our lives as investigators and as pediatricians.

RESILIENCE

The cell wall of *C. albicans* is 200 nm thick, thicker than *Saccharomyces* or virtually any other organism save plants (Fig. 8). Its six layers of chitin, β -glucans, and mannoproteins present a nearly impenetrable barrier to our pharmacologic armaments. This is one tough fungus. And *Candida* is a clever opportunist as well, seizing the breaches in host defense—the newborn, the neutropenic, the immunocompromised—for its attacks. And now I speak to the fellows and junior faculty in the audience, to the 400 of you who packed our educational symposia and who are the life's blood of this Society. If you as young investigators are to succeed in research for children, you too must toughen up and seize the opportunities. Don't be afraid to make mistakes. If it's good, it can be made better; if it's wrong, it can be righted. Don't be deterred by rejection. My papers are rejected, and my grants aren't always funded. When faced with adversity, act like a yeast. Take the punch; it's how you come back that counts.

TRANSFORMATION

One of the most bewildering mysteries in *Candida* is its uncanny ability to transform, from yeast to budding daughter cell to pluripotent hyphae (Fig. 9). In *Candida*,

Figure 7. Model for evolution of integrins.

Figure 8. Schematic diagram of the seven layers of the candidal cell wall (reprinted from Shepherd MG 1987 Crit Rev Microbiol 15:7-25, with permission of the publisher).

Figure 9. Figure is reprinted from Borgers M 1993 Morphological aspects of fungal dimorphism. In: Vanden Bossche H, Odds FC, Kerridge D (eds) Dimorphic Fungi. Plenum Press, New York, p 124, with permission of the publishers.

this metamorphosis endows the organism with an unequaled pathogenic potential. Transformation also occurs in pediatrics, as these pictures show: the house staff of the pediatric service at Johns Hopkins, 1955—and here, for you archivists, is Dr. Mary Ellen Avery, one of my first mentors. Just 20 years later, we see Dr. Avery with her house staff at Boston Children's Hospital, 1975. What changes have ensued: from uniforms (or the lack thereof) to hairstyles and even to more subtle transformations. This era was one of unprecedented growth in academic medicine, in subspecialty training, and in the inclusion of women and minorities. Now, of course, we confront the next 20 years, and the future looks dark to many.

- What will we do with the 13 million children who have no health insurance? Tell them to wait until they are 65?
- What will we say to the hundreds of thousands of children who are hungry by day and homeless at night? Tell them they just don't count?
- What will we do to convince government, the press, and the lay public of the value of research for children?

We will transform ourselves yet again; we will become more adept, more versatile in the most adverse of circumstances; and we will say "Look with us at these faces."

Hyaline membrane disease—a thing of the past. Tuberculosis treated. Leukemia left behind on a summer afternoon. Asthma off your back. Hemophilus influenzae vanquished. How high we have soared on the scientific imagination! These faces, and the faces of thousands of children like them, are our victories, hard won through pediatric research and through the contributions of members of our Society. We celebrate our successes. But let us be sure to give all our children the gift of flight. Let us watch every one soar above the plane of poverty, ignorance, and disease into that brilliant blue expanse of possibility. And how do we do that? Take a lesson, once again, from yeast.

COHESION

These are single yeasts: isolated, insular (Fig. 10, *left panel*). I can kill them easily, just by changing the environment. A little amphotericin, a new imidazole, and

Figure 10. The *left panel* is reprinted from Kwon-Chung KJ, Bennett JE (eds) 1992 Medical Mycology. Lea and Febiger, Philadelphia, PA, plate 13.21, p 313; the *right panel*, from Beneke ES, Rippon JW, Rogers AL (eds) 1984 Human Mycoses. Upjohn Company, Kalamazoo, MI, plate 130, p 61, with permission of the respective publishers.

cellular synthesis breaks down completely. But this is yeast cohesion (Fig. 10, *right panel*), and this skein, this uncompromising network, is much more difficult to disrupt. So it must be with pediatrics. It cannot be my HMO against your hospital, your department against mine, private against academic. Given the upheaval and conflict in the medical profession, there will be many opportunities for divisiveness, anger, and confusion. But we must look beyond the immediate arena to the larger vista, and our loyalties must not deviate. The overarching vision is not power, prestige, or position. The vision is children. Your children, my children, the children we teach and the children we treat: sons and daughters *all*. We are a colony, a community of pediatricians with a common goal: that science must bring hope to children, and that we, as their champions, must speak with one voice for those without.

The words are those of Perri Klass (11), from her book, *Other Women's Children*, but the march and the battle and the victory must be ours:

"Imagine them all getting better. All through the hospital, the children get better. Their breathing gets easier, they poke their heads out of the oxygen tents and take deep breaths of room air. They pull the i.v.s out of their hands, their feet, their chests, their heads. No one needs antibiotics; the evil bacteria are dead. No one needs intravenous nutrition; they are all hungry for milk, baby food, hamburgers. Imagine them strong enough to jump from their beds and swarm through the corridors. . . . Imagine the corridors full of children, the big ones running, the toddlers toddling, the little ones crawling. No more fevers, no more wheezing, above all; no more pain . . . And in every room, on every ward. Imagine them pouring out the front doors, the big ones now carrying the babies, reaching down to hold the hands of the toddlers, the whole pack disappearing down the street, leaving the hospital, with its humming lights and beeping monitors; leaving it behind."

Resilience. Transformation. Cohesion. That message leaps across one billion years of evolution. That is the beauty of biology, the power of science, and the glory of pediatrics. Children first. And we must see it done.

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