

Response from Gale *et al.*

Dr Calderone raises several minor criticisms of our recent publication in *Science*¹. First, he believes that we are incorrect in saying that *Candida albicans* causes invasive disease in patients infected with HIV. While it is true that *C. albicans* rarely causes bloodborne disease in HIV-infected patients, it often causes a painful, invasive mucosal infection in these patients.

Second, Calderone wants to see additional data from the virulence assays, including the tissue load of microorganisms and the histopathology. We have performed histopathology on mice injected with the *C. albicans int1* mutant strains and find that deletion of both copies of *INT1* results in fewer mouse deaths but prolonged tissue survival².

Third, as noted by Calderone, the morphology of the cell is one of several determinants in the adhesive ability of *C. albicans*. This

was not the case for the *C. albicans* strains subjected to our adhesion assay: by direct microscopic visualization of the cell monolayers during the assay, we determined that all of the *C. albicans* strains were in the blastoconidial form during the entire adhesion assay. The *C. albicans* cells were incubated with the cell monolayers at 37°C for only 30 min: a length of time deliberately chosen to avert the induction of hyphae at this temperature. The details of the adhesion assay are described by Gustafson *et al.*³ and are also referred to in our recent paper¹. We agree that this strengthens our claim that Int1p functions as an adhesin in *C. albicans*, as there should be less variability of results between cells sharing the common blastoconidial morphology.

In addition, Calderone raises two major issues, which we will address here. First, he objects to our use of human cervical epithelial (HeLa) cells as a model for candidiasis. We agree that HeLa cells are not the only cell line that should be used to define potential adhesins and we are currently investigating the role of Int1p in adhesion of *C. albicans* to Caco-2 cells *in vitro* and in epithelial cell models *in vivo* [K. Kinneberg *et al.* (1998) Am. Soc. Microbiol. 98th Gen. Meeting, Atlanta, GA, USA, Abstr. F-29]. It is interesting to note that when the cervix is cultured or biopsied, *C. albicans* is present ~20–30% of the time, which is similar to the incidence of *C. albicans* in vaginal cultures^{4,5}. Therefore, HeLa cells are an appropriate epithelial cell model. As noted by Calderone, endothelial cell models are also important to study with regard to candidal adhesion: once *C. albicans* invades the mucosal barrier, it probably adheres to and invades the endothelial cell layer and, from there, disseminates into the bloodstream. However, when *C. albicans* invades blood vessels, the opportunity to prevent systemic disease is probably lost.

Second, Calderone is particularly troubled by the lack of sequence homology between vertebrate integrins and *INT1*. *INT1* was

cloned using cDNA probes from a human integrin, thus giving rise to its name. *INT1* encodes a protein with 18% overall identity to the α M integrin. Interestingly, human α M and human α 1 only share 32% identity. As previously reported⁶, Int1p contains two partial EF-hand (potential divalent-cation-binding) motifs; human integrin α M contains three partial EF-hand motifs. In addition, Int1p contains the sequence KKRFK at its carboxyl terminus, which has 67% identity and 100% similarity at the protein level, and 78% identity at the nucleotide level, with the α M carboxy-terminal sequence KVGFFK, which was used for the secondary screens in the cloning of *INT1*.

More importantly, Int1p appears to share some functional similarities with integrins: *Saccharomyces cerevisiae* expressing *INT1* binds the anti-human integrin (α M) antibody OKM1, has increased aggregation and undergoes a change in morphology⁶. In addition, Int1p is a surface protein that mediates adhesion¹. Because *C. albicans* presumably expresses numerous adhesins that complicate the detection of changes in adhesion with removal of just one protein, we expressed *INT1* in *S. cerevisiae* (a nonadherent, usually nonpathogenic, yeast) and studied the effect of *INT1*, in isolation, on adhesion. We showed that the adhesion of *INT1*-expressing *S. cerevisiae* to HeLa cell monolayers was tenfold more than that of wild-type *S. cerevisiae* and that the anti-Int1p antibody, UMN13, blocks this adhesion by approximately two-thirds¹. Calderone questions the use of UMN13 rather than another anti-Int1p antibody (UMN12), noting that blockade of adhesion with UMN12 would have strengthened the contention that Int1p is an integrin. As shown in Fig. 1, incubation of *S. cerevisiae* with UMN12 reduces adhesion to HeLa cells to almost the same extent as does UMN13. Although the function of Int1p as an adhesin is probably more important than its classification as an integrin, we do see that UMN12, an antibody

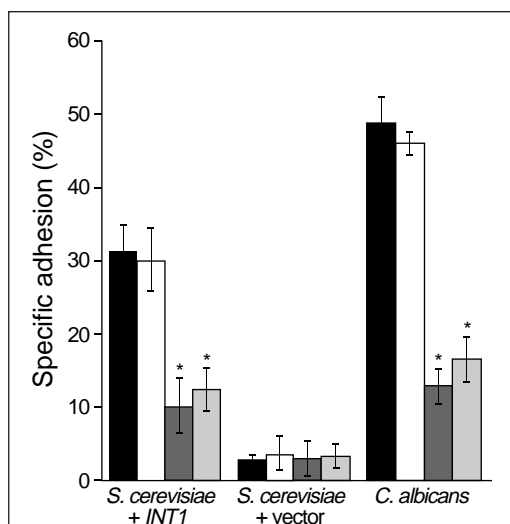


Fig. 1. Adhesion of *Saccharomyces cerevisiae* expressing the *Candida albicans* gene *INT1* to HeLa cell monolayers. Results are reported as specific adhesion and are expressed as the mean \pm standard error of the mean. The asterisks represent a significance of $P < 0.005$. Black bars represent adhesion of yeast cells in the presence of buffer alone; white bars represent adhesion of yeast cells incubated with non-immune rabbit immunoglobulin G; dark gray bars represent adhesion of yeast cells incubated with the anti-Int1p antibody UMN12; and light gray bars represent adhesion of yeast cells incubated with the anti-Int1p antibody, UMN13.

raised against a peptide encoding one of the EF-hand motifs and located near the putative ligand-binding domain of Int1p, blocks adhesion of *INT1*-expressing *S. cerevisiae* to HeLa cell monolayers.

Expression of *INT1* confers an adhesive ability, more clearly observed in *S. cerevisiae* but also seen in *C. albicans*, and, in addition, contributes to morphogenesis in both yeasts. In this respect, *INT1* seems to fit the vertebrate integrin paradigm. However, although Int1p shares some func-

tional similarities with integrins, we do not consider it to be an integrin in the classic sense and hence we refer to it as 'integrin-like'. Considering the evolutionary distance between vertebrates and *C. albicans*, we think that the function is more important than the name.

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Letter

Pathogenesis of chronic bacterial infections

Chronic infectious disease is frequently perceived in a unitary fashion from the cellular point of view; i.e. all cells in a chronically infected host harbor the infectious agent, and the kinetics and result of infection are essentially the same in each infected cell. An alternative hypothesis is that these diseases involve simultaneous interrelated cycles of chronic and acute infections between a limited number of cell types. Infection of one cell type may be chronic and latent, produce little cytopathology and only rarely lead to the release of infectious units. Infection of other cell type(s) may be more acute, cause rapid and extensive cytopathic effects and the release of large numbers of infectious units. Signs and symptoms of chronic disease in the host are often dominated by damage to the cell type that exhibits the more acute and cytopathic response to infection.

Interrelated cycles of chronic and acute patterns of infection in different cell types have been most clearly delineated in HIV infection¹. Chronically HIV-infected dendritic cells and other mononuclear phagocytes show little cytopathic effect, whereas the raging acute HIV infection of activated CD4⁺ T cells leads to their destruction. A somewhat similar, but less completely defined, pattern occurs in herpes

simplex infections². In this case, the occasional production of infectious virions by chronically infected neurons is associated with the more acute and cytopathic mucocutaneous infection of epithelial cells. In malaria caused by *Plasmodium vivax*, a symptomatic, cytolytic, intraerythrocytic infection is superimposed on an essentially asymptomatic chronic hepatic infection³.

Whether a similar pattern of interrelated cycles of infection and response of different cell types is also involved in the pathogenesis of chronic bacterial diseases is unclear. Infection of macrophages by *Mycobacterium leprae* is apparently not highly cytopathic in lepromatous leprosy, and direct infection of Schwann cells appears to be at least partly responsible for peripheral nerve dysfunction in this disease^{3,4}, but evidence for a differential response to infection by these two cell types is limited⁵. Tuberculosis presents an even more speculative case for interrelated acute and chronic infections of different cell types, as the types involved have not been fully defined. However, the relative inability of mice to control the growth of *Mycobacterium tuberculosis* in the lung, as compared with in the liver and spleen^{6,7}, is consistent with an indolent infection in a cell type localized to the lung

(e.g. pneumocytes or alveolar macrophages) with the occasional release of viable bacteria leading to active new infections in resident and recruited inflammatory macrophages. In both these diseases, the role of interrelated cycles of infection in pathogenesis is likely to be amplified by, and be in addition to, that played by the hypersensitivity of the host to mycobacterial antigens.

Recognition of the role that dual patterns of infection may play in pathogenesis of chronic diseases caused by intracellular bacteria is likely to suggest new approaches to their study and lead to a more refined view of chronic infectious disease at the cellular level.

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