New hope for fungal vaccines?

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Researchers have generated the first live fungal vaccine to be produced using recombinant DNA technology[1]. The vaccine, which was developed by Bruce Klein and his colleagues at the University of Wisconsin-Madison (WI, USA), has been produced for immunization against *Blastomyces dermatitidis*.

The systemic dimorphic fungus *B. dermatitidis* (Fig. 1) causes the often-fatal lung infection, blastomycosis, and is one of the principal systemic mycoses of humans and animals worldwide. After inhalation of the spores, the fungus is converted into a pathogenic yeast. This disease is characterized by fever, coughing, general malaise and weight loss which, in more severe cases, can lead to a chronic and progressive pneumonia and sometimes death. In approximately half of infected individuals, the infection disseminates to other organs such as the skin, bone and the brain. Treatment with oral antifungals can be effective if diagnosis is made early, but therapy often lasts for 6–12 months and can be associated with side effects. In more severe cases, patients can require hospitalization and therapy with intravenous antifungals (such as amphotericin B), which have a much higher incidence of side effects and can cause severe toxicity.

Problems of live fungal vaccines

Few experimental antifungal vaccines have been developed, and none are available for human use so far because of the complexity of these organisms, says Dennis Dixon, Chief of Bacteriology and Mycology at the National Institute of Allergy and Infectious Diseases (NIAID), the sponsors of the project. For example, the *B. dermatitidis* genome has 25 million base pairs, compared with 4.6 million for *Escherichia coli* and 10,000 for HIV. Furthermore, Dixon points out that, being eukaryotic organisms, 'there is much similarity between the processes in humans and fungi, making selective targets difficult to find. Klein adds that, 'fungi are much harder to manipulate than bacteria because they have a much thicker cell wall, making effective gene transfer systems harder to develop'. There has also been relatively little interest in producing an antifungal vaccine prior to the recent increase in the incidence and profile of fungal infections, mostly in immunocompromised patients such as those with HIV, cancer or in intensive care.

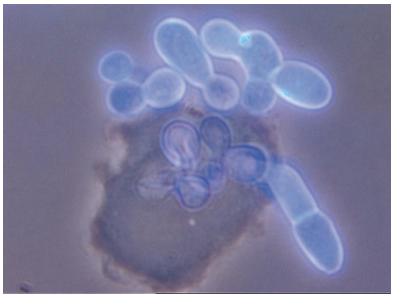

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Figure 1. The fungus, *Blastomyces dermatitidis* adhering to macrophages.

Some attempts have been made previously to develop live attenuated vaccines using spontaneous or induced variants for fungi such as *Coccidioides immitis*[2], *Trichophyton verrucosum* (for use against ringworm in cattle)[3] and *Candida albicans*[4]. 'The advantage of developing a live attenuated vaccine is that it does everything that a wild-type organism would do,' says Klein. 'However, what distinguishes past studies from ours is that spontaneous or induced variants can be unstable and are likely to revert spontaneously to the disease-causing form, such as is sometimes seen with the current polio vaccine,' he warns. 'Our vaccine is genetically engineered and we have conducted studies to show that the targeted mutation is stable and does not revert,' he continued.

WI-1 (a surface protein adhesin on *B. dermatitidis*) has previously been shown to be an immunodominant antigen that evokes a humoral and cell-mediated immune response in infected humans[5], dogs[6] and mice[7]. In a murine model of lethal pulmonary blastomycosis, Klein and colleagues have previously shown that WI-1 immunization can prolong the survival of mice, but only by 1–2 weeks.

However, after targeted disruption of the WI-1 adhesin gene (WI-1 knockout strain no. 55), they found that the yeast could not establish a lethal infection in mice[8]. 'This was a big step forward for us,' says Klein. 'We observed the knockout strain sequestered inside granulomas in the lungs of infected animals and so we wondered if the host was being immunized to the pathogenic organism.' This therefore prompted the investigation of the knockout strain as a vaccine for pulmonary blastomycosis[1].

WI-1 knockout strain as a vaccine

The efficacy of the WI-1 knockout strain no. 55 was tested in mice and its virulence monitored periodically throughout the experiments by measurement of surface α-(1,3)-glucan and WI-1. C57BL/6 and BALB/c mice were vaccinated using two injections of strain no. 55 intranasally or subcutaneously, or three injections using multiple routes (intranasally, subcutaneously and intravenously) at two-week intervals. Mice were then challenged with the parent wild-type strain, ACC 26199.

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All routes of administration significantly reduced lung colony-forming units (CFU) of the organism, but the subcutaneous and combined routes of administration were the most effective, reducing CFU levels by at least three logs, 2–3 weeks post-infection. In survival studies, 80% of the subcutaneously immunized mice survived the lethal challenge, as did 50% of those immunized by the combined route, as measured by survival 128 days post-infection. Furthermore, the immunized mice were cross-protected against infection with nonisogenic strains from other parts of the States, showing a 2–6-log reduction in lung CFU compared with non-immune controls. Further studies showed that the vaccine induces a polarized type 1 cytokine profile tightly linked with vaccine-induced resistance to *B. dermatitidis* and leads to the production of interferon-γ (IFN-γ) in response to *in vitro* antigen stimulation.

Identification of key antigens

'We then wanted to know what antigens are being recognized within the fungus with the hope that we might be able to produce a subunit vaccine, therefore reducing the risks of using a live attenuated organism,' says Klein. Three crude antigen preparations, YSP (yeast surface proteins), YCE (yeast cytosol extract) and CW/M (cell-wall membrane) were prepared from strain no. 55 and tested for their vaccination potential. CW/M was the most effective at reducing the level of lung infection and invoked the strongest IFN-γ and interleukin IL-2 response *in vitro* on stimulation with antigen. Klein suggests that this might be because of the presence of a greater number of protein antigens or of protective components in CW/M compared with YSP and YCE. In a mouse model of chronic pulmonary infection (through repeated inoculation with fewer organisms), YCE and CW/M protected more than 70% of mice from death, suggesting the potential of the vaccine for the chronic infection blastomycosis.

'This is a very nice proof-of-principle study that identified what makes the fungus pathogenic and then showed that removal of the gene blunts virulence,' says Dixon. 'However, this needs to be much more extensively studied before it is time to consider a live fungal vaccine for human use,' he cautions. Dixon suggests that: 'The natural incidence of blastomycosis in dogs presents an excellent opportunity for designing safety and efficacy studies in a relevant mammalian host with a real potential to benefit from the prevention strategy represented by this work. Additionally, WI-1 itself might be a viable candidate for the vaccine as it would circumvent concerns over the stability of the knockout mutant.'

Future hopes

Klein now wants to know whether this vaccine might work in species other than mice. 'Blastomycosis is much more prevalent in dogs than in humans and therefore provides a good opportunity to not only create a useful vaccine for dogs but also to test the effects of the vaccine on another species,' says Klein. The team is currently trying to optimize the dose for vaccination in dogs and preliminary results look promising. If these studies are successful, they hope to extend the studies to dogs in the community.

Klein also hopes that this work will encourage research into the development of similar vaccines for other more prevalent fungal diseases. They are now investigating whether *B. dermatitidis* shares any antigens with other pathogenic fungi. Such fungi include *C. immitis* (which caused an epidemic of San Joaquin Valley Fever in California in 1992), and *Histoplasma capsulatum* (which causes histoplasmosis in most of the population by the age of 20 in Ohio and Mississippi River Valley), as well as more prevalent, but more distant fungal species such as *Aspergillus.*

Finally, Klein and his team are investigating the immunological mechanisms involved in the induction of immunogenicity by the vaccine and are hoping to publish some 'unexpected' results in the next few months. Dixon hopes that, 'Other researchers in the field will be encouraged by what Klein and his team have accomplished, and this should lead to some exciting developments in fungal vaccinations in the future.'

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