Box 1. Outstanding questions

- How does the composition of AMPs that *Salmonella* might encounter in the host intestines and macrophages, and that could activate PhoP–PhoQ virulence program, change throughout infection?
- Which other host molecules can activate PhoP–PhoQ in *Salmonella*, and what is the molecular mechanism by which PhoQ can accommodate a wide variety of AMPs or other host-defense molecules?
- Which specific virulence traits are activated by AMP or other host molecules in a PhoP–PhoQ-dependent manner in *Salmonella*? How do these traits translate into disease?
- Are two-component regulatory systems activated by host molecules in other pathogens during infection, and are they important for disease progression?
- How can we develop AMPs or other host-molecule-derived therapies with low risk of triggering pathogen virulence or drug resistance?

Undoubtedly, finding the balance between AMP immunomodulation and resistance induction will be the focus of future work in AMP drug discovery.

The idea that innate immune molecules such as AMPs stimulate bacterial virulence is both exciting and daunting. AMP-triggered activation of a powerful system such as *Salmonella* PhoP–PhoQ means that these pathogens can synchronize their physiology both with their intracellular environment and with specific molecules of the host immune response. To control such pathogens in the clinical setting, stringent examinations of the mechanism by which AMPs or other molecules trigger virulence must be completed, and discovering how these virulence traits contribute to disease in appropriate model systems will be crucial (Box 1). The implications of such work will heavily influence the fields of pathogenesis and drug research as we attempt to develop AMPs as safe and effective novel therapeutics.

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Disclosure statement

B.B.F. is a co-founder of Inimex Pharmaceuticals Inc.

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Polysaccharide-containing conjugate vaccines for fungal diseases

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The recognition that antibodies are effective against fungal pathogens has spawned interest in developing vaccines that elicit antibody-mediated protection. Recently, a novel polysaccharide–protein conjugate vaccine that uses the algal antigen laminarin was shown to elicit antibodies to β-glucan in fungal cell walls and to mediate protection against both

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experimental candidiasis and aspergillosis. Remarkably, vaccine-induced antibodies manifested direct antifungal effects, suggesting that vaccine efficacy might not require cellular or other components of the immune system. The description of a vaccine that could protect against various fungal pathogens opens exciting new dimensions in the search for approaches to control fungal diseases.

Introduction

In the 1990s, a paradigm shift changed our view of the relative importance of cellular and antibody immunity in protection against fungal diseases. The prevailing view had been that host defense against fungi was the purview of cellular immunity without a significant role for antibody-mediated immunity (AMI) [1]. However, this view was supported largely by negative data, rather than direct proof. The problem was that it had been difficult to establish a role for AMI consistently in studies using available antibody reagents, which were limited to immune sera, possibly because such sera contained both protective and non-protective antibodies and/or insufficient amounts of protective antibody. A breakthrough occurred with the application of monoclonal antibody (mAb) technology to development of antibodies to fungi [2]. The availability of mAbs made it possible to conduct studies using defined, homogenous reagents, which led to the unexpected discovery of protective and non-protective antibodies to various fungi. Hence, the problem was not that antibodies had no role in mediating protection, but rather that neither infection nor immunization reliably produced a predominance of protective antibodies. Subsequently, AMI was shown to be effective against most major fungal pathogens. The discovery that antibodies could mediate protection against fungi was crucial to the fungal vaccine field, because it established the feasibility of designing vaccines that elicit AMI. Because currently available vaccines are believed to work by eliciting AMI [3], the effectiveness of AMI against fungi means that fungal vaccines could, in theory, be designed based on the successful precedents of vaccines for bacteria and viruses.

Antibodies can be powerful molecules against fungal pathogens

The realization that protective AMI could be marshaled against fungi immediately stimulated interest in the generation of vaccines that could induce protective antibodies [4]. Because pathogenic fungi have cell walls and capsules composed of polysaccharides, these antigens were logical targets; however, polysaccharides are known to be poorly immunogenic. One approach to enhance the immunogenicity of polysaccharide antigens is to link them to proteins by a covalent bond. Polysaccharide–protein conjugate vaccines have been remarkably effective in reducing the burden of disease caused by *Haemophilus influenzae* and *Streptococcus pneumoniae* in children. Early attempts to produce conjugate vaccines for fungi were not successful, perhaps because heterogeneous polysaccharide preparations were used. However, by the mid-1990s polysaccharide–protein conjugate vaccines consisting of fungal antigens were shown to be effective against both *Cryptococcus neoformans* and *Candida albicans* [5,6]. Furthermore, the concept was taken forward with the development of protective experimental vaccines based on peptide mimotopes of polysaccharide-binding antibodies that elicit protective AMI [7].

A polysaccharide–protein conjugate vaccine with broad activity against different fungi

Torosantucci *et al.* have recently reported that a vaccine composed of laminarin and the protein-carrier diptheria toxoid CRM197 was highly immunogenic and protected mice against two fungal pathogens, *Candida albicans* and *Aspergillus fumigatus* [8]. Laminarin is an algal β-glucan that is poorly immunogenic as an unconjugated moiety. The laminarin conjugate vaccine has several novel features. First, the polysaccharide antigen is from algae, which belong to a different biological kingdom from fungi. Hence, the vaccine works by eliciting cross-reactive antibodies and seems to defy the dogma that vaccines protect by eliciting microbe-specific responses. Second, the vaccine was protective against two very different types of fungal pathogens. Because β-glucan is common in fungal cell walls, the vaccine could protect against other pathogenic fungi. The concept of a vaccine that protects against an entire class of pathogens would be a revolutionary advance that could guide vaccine research away from efforts to elicit pathogen-specific immune responses and towards the currently heretical thought of stimulating protection through cross-reactive antibodies. Although vaccine efficacy based on cross-reactive immunity is not a new idea, as exemplified by precedent of vaccination against smallpox using Vaccinia virus [9], the laminarin–CRM197 vaccine takes the concept into new territory. The use of an algal antigen, a structural homolog derived from a different, non-microbial, genus to elicit antibodies that in turn can protect against two phylogenetically distant fungi represents an unprecedented application of the principle of protective cross-reactivity in vaccine design. The apparent success of this vaccine in animals opens new possibilities for antigen discovery and vaccine design. A third novel feature of this vaccine is that the antibodies elicited by laminarin appear to be directly antimicrobial, which could obviate or reduce the need for other components of the immune system in vaccine efficacy. This is particularly important because patients at high risk for candidiasis and aspergillosis are often severely immunocompromised with deficiencies in both innate and adaptive immunity, and the ability of antibodies to be directly antimicrobial means that the immunoglobulins would be active despite these immune defects.

A potential vaccine for prevention of human candidiasis and aspergillosis

The laminarin–CRM197 vaccine was protective against systemic and mucosal candidiasis in mice and rats, respectively [8]. Hence, the vaccine is effective in at least two mammalian species that differ significantly in their immune response and susceptibility to fungal pathogens. In vaginal candidiasis experiments the vaccine was

administered intra-vaginally, demonstrating its potential usefulness for mucosal immunization [8]. Because systemic and mucosal hosts defense against *C. albicans* employ different immune mechanisms, the finding that the vaccine induced protection against both forms of disease suggests that its efficacy depends on direct immunoglobulin antimicrobial effects [10] and/or that it induces antibodies that mediate protection by different mechanisms in different compartments. Antibodies that mediate direct effects against fungi by binding to cell-wall components have been described [11,12]. Alternatively, because fungal β-glucans can be pro-inflammatory, certain laminarin-induced antibodies could function as immunomodulators, as described for other microbes [13]. Another interesting aspect of vaccine-mediated immunity is that the optimal mediator of protection for passive vaccination differs depending on the infection model, whereby serum is more efficacious than mAbs against systemic disease, but mAbs are more efficacious than serum against mucosal disease. In addition, although IgM was not believed to contribute to the efficacy of passively administered immune serum, it could have contributed to the protection conferred by active vaccination, which induced titers of IgM and IgG that were nearly equivalent to those of laminarin. Hence, the success of the vaccine opens new possibilities in understanding vaccine mechanisms and antibody efficacy in immune and naïve hosts.

The availability of a laminarin–CRM197 vaccine, or of a similar derivative, that reduces the incidence of candidiasis and aspergillosis would be a major advance in medicine, because these fungal diseases are very common in patients with impaired immunity and are associated with high morbidity and mortality. One problem with polysaccharide–protein conjugate vaccines is that they are often poorly immunogenic in individuals with impaired immunity. However, a laminarin–CRM197 vaccine could be given to patients who are about to undergo surgery, organ transplantation and immunosuppressive therapy (e.g. before the onset of immunosuppression) to elicit pre-existing AMI that would lessen the susceptibility to fungal diseases. Furthermore, such a vaccine could be used to generate potent hyperimmune sera for passive immunization in individuals not responding to the vaccine.

Concluding remarks

The vaccine described by Torosantucci *et al.* [8] illustrates the progress that can be made when investigators are freed from the conceptual limitations of categorizing the effectiveness of immune responses according to pathogen type and the cellular–humoral immune divide that has plagued immunology for over a century. Despite the novelty of this vaccine, it is worth considering it with caution. Broadly effective vaccines could alter the balance between host and microbial flora and might have unanticipated untoward effects. Similar to other conjugate and subunit vaccines, laminarin might induce antibody responses that are not comparable in normal and impaired hosts; future studies need to establish the molecular genetics of antibody responses to β-glucan in both humans and experimental animals (Box 1). Furthermore, it is important to dissect the basis of cross-reactivity carefully because it could represent the presence of shared structures in fungal cell walls or the generation of antibodies with molecular structures that enable reactivity with multiple antigens. The latter would raise concerns about the potential for self-reactivity. The laminarin–CRM197 vaccine reveals how a paradigm shift in understanding the role of AMI in host defense led to a new experimental vaccine. Additionally, this vaccine could further catalyze a paradigm shift in vaccine design by encouraging research efforts to identify mechanisms by which cross-reactive antibodies mediate protection and contribute to host defense. Hence, this vaccine is both the product of a revolutionary thinking and itself revolutionary, because it demonstrates the hitherto unrecognized potential of cross-reactive immunity in protection against different classes of pathogens. Nevertheless, this vaccine is remarkable because it provides proof-of-principle for the notion of a broadly protective vaccine and opens possibilities unimaginable a few years ago.

Box 1. Outstanding questions

- By what mechanism do antibodies to fungal cells inhibit their growth?
- Are the specificity and molecular genetic derivation of laminarin-binding antibodies that mediate protection against *C. albicans* and *A. fumigatus* the same?
- Do laminarin-binding antibodies that react with *C. albicans* and *A. fumigatus* also react with other fungi?
- Do human laminarin-binding antibodies also bind to fungal cell-wall antigens?
- What are the molecular structures responsible for the antigenic mimicry resulting in cross-reactivity between antibodies to laminarin and β-glucans?
- Can the principle of cross-reactivity be applied to vaccine design for microbes with other ubiquitous antigens?
- Are vaccines that elicit broadly cross-reactive antibody responses safe?

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