Review Article

Aspergillus vaccines: Hardly worth studying or worthy of hard study?

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Abstract

Vaccines rank among the greatest advances in the history of public health. Yet, despite the need, there are no licensed vaccines to protect humans against fungal diseases, including aspergillosis. In this focused review, some of the major scientific and logistical challenges to developing vaccines to protect at-risk individuals against aspergillosis are discussed. Approaches that have shown promise in animal models include vaccines that protect against multiple fungal genera and those that are specifically directed to Aspergillus. Advances in proteomics and glycomics have facilitated identification of candidate antigens for use in subunit vaccines. Novel adjuvants and delivery systems are becoming available that can skew vaccine responses toward those associated with protection. Immunotherapy consisting of adoptive transfer of Aspergillus-specific T cells to allogeneic hematopoietic transplant recipients has advanced to human testing but is technically difficult and of unproven benefit. While progress has been impressive, much work still needs to be done if vaccines against aspergillosis are to become a reality.

Key words: aspergillosis, fungal vaccines, immunotherapy, allergy, allogeneic hematopoietic transplant.

Introduction

Aspergillus is ubiquitous in the environment; it is estimated that individuals typically inhale hundreds of conidia a day. In the vast majority of individuals, host defenses are able to easily contain the fungus, and the near constant encounters with the fungus are harmless occurrences. However, Aspergillus causes a wide spectrum of acute and chronic diseases in persons with compromised host defenses. Thus, in persons with severe immunocompromise, particularly those with impaired neutrophil function and recipients of allogeneic transplants, inhaled conidia can germinate into hyphae, which then invade lung tissue and eventually disseminate to other organs. The burden of invasive aspergillosis (IA) is estimated at over 200,000 persons per year. In patients who are atopic or have cystic fibrosis, sensitization to Aspergillus can result in allergic manifestations including severe asthma with fungal sensitization (SAFS) and allergic bronchopulmonary aspergillosis (ABPA). An estimated 5 million people have ABPA. Persons with preexisting cavitary lung disease, such as might occur due to tuberculosis or sarcoidosis, are at risk for developing colonization of the cavities with Aspergillus. While these so-called
aspergillomas are often asymptomatic, life-threatening complications, particularly hemoptysis, can occur.

Hundreds of species of Aspergillus have been described although the vast majority of human infections are caused by a handful of species including A. fumigatus, A. flavus, and A. niger. Many of the antifungals in clinical use lack intrinsic activity against Aspergillus, and for those that have anti-mould activity, resistance has become a widespread concern. Tests to diagnose invasive aspergillosis exist, but they generally lack sensitivity; this can result in fatal delays in diagnosis. While the substantial morbidity and mortality associated with aspergillosis have prompted urgent calls for new drugs and diagnostics, they have also stimulated research into vaccines to protect at risk patients and to treat those with disease. Here, I review the obstacles to vaccine development (and their potential solutions), define populations that could benefit from vaccinations, and provide examples of promising approaches. Due to space limitations, many excellent studies could not be included. A comprehensive review of Aspergillus vaccines including immunotherapeutic approaches has recently been published.

Are Aspergillus vaccines feasible?

As noted above, a large number of individuals are at risk for developing aspergillosis and thus could presumably benefit from vaccination. In this section, the numerous factors which hinder the development, testing and marketing of Aspergillus vaccines are discussed along with possible solutions (Table 1). Patients with aspergillosis have a wide range of immunological abnormalities. Those at risk for invasive aspergillosis generally have severe immunocompromise that greatly limits their response to vaccination. This includes defects in innate (particularly neutropenia during periods of chemotherapy) and adaptive defenses. Possible solutions include novel vaccine formulations containing potent adjuvants to elicit protective responses. Patients who will be immunosuppressed in the future, such as those on transplant waiting lists, could be vaccinated prior to transplant when their immune response is relatively intact. For those receiving allogeneic hematopoietic transplants, protective donor lymphocytes could be transplanted. On the other end of the spectrum, those with allergic manifestations have robust but dysregulated immune responses. In this patient population, a successful vaccine strategy could be to dampen or redirect the nature of the immune response such as by shifting the bias from Th2 to Th1.

Most candidate Aspergillus vaccines are being tested in mice. Mice and humans diverged approximately 65 million years ago, and while the basics of the immune system are quite similar, there are key differences. In addition, most studies use inbred mice that lack the genetic diversity of “outbred” humans. Perhaps more importantly, laboratory mice live in filtered cages and thus have no natural exposure to Aspergillus. This contrasts with humans who are repeatedly exposed to airborne fungi. Possible solutions include the use of multiple animal models including humanized mice, modeling natural exposure conditions by continuous challenge, and conducting in vitro human testing.

Aspergillus species and Homo sapiens are both eukaryotes. As such, many potential Aspergillus vaccine candidates have significant homology to human proteins and may either not elicit robust immunological responses or could trigger autoimmunity. However, with the human genome sequenced, such proteins can be avoided or the homologous portions excluded from vaccines. Another issue is that fungi extensively glycosylate many of their proteins, particularly the cell wall proteins that might be the most promising vaccine candidates. Moreover, the pattern of glycosylation can vary as a function of fungal species. If protective antibody responses are to the glycan portion of the molecule, then expressing the protein in a prokaryotic vector or even a yeast vector would not be expected to work.

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<th>Obstacle to Vaccine development</th>
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One option is to incorporate native glycoprotein into the vaccine.

Criteria for diagnosing allergic manifestations of aspergillosis and subsequent responses to treatment are fairly well established. However, establishing a diagnosis of invasive aspergillosis can be difficult due to the insensitivity of diagnostic tests. As a consequence, the majority of patients enrolled in clinical trials have possible or probable invasive aspergillosis, rather than proven disease. This, combined with the increasing use of anti-mould prophylaxis in high risk groups will necessitate recruiting large numbers of patients for Aspergillus vaccine trials. Nevertheless, numerous comparative studies of antifungal regimens for invasive aspergillosis have been successfully conducted, and similar studies with clinically meaningful endpoints could be performed for vaccines. Moreover, improvements in diagnostics could facilitate identification of probable and proven cases of aspergillosis.

Aspergillus vaccines
Pan-fungal vaccines
Aspergillus vaccines can be subdivided into four somewhat overlapping categories: pan-fungal, crude, subunit, and therapeutic. Representative examples of each of these categories will be discussed as well as considerations regarding delivery systems and adjuvants. Pan-fungal vaccines take advantage of shared antigens possessed by fungi to protect against many medically important fungal species genera. Such vaccines may be more likely to attract commercial interest because, if successful, they will protect against many different mycoses. The Cassone laboratory conjugated β-1,3-D-glucan (in the form of laminarin) to diphtheria toxoid. Mice immunized with the conjugate glycoprotein developed robust antibody responses to β-1,3-D-glucan and were protected against challenge with several different fungi including Aspergillus. Similarly, Clemons et al. were able to protect mice from five genera of fungi including the fumigatus species of Aspergillus by vaccination with heat-killed Saccharomyces cerevisiae yeast. Protection did not appear to be antibody-dependent. One possible mechanism is stimulation of memory innate defenses (also known as “trained immunity”); β-1,3-D-glucans have been shown to induce epigenetic reprogramming and non-specific protection against infections. Wuthrich et al. discovered a T-cell epitope in the protein calnexin that was highly conserved across members of the Ascomycota (the fungal phylum that contains Aspergillus). The investigators demonstrated that calnexin localized to the surface of the fungi and that vaccination of mice with calnexin resulted in expansion of antigen-specific CD4+ T cells. However, calnexin-mediated protection against Aspergillus was not studied. Proteomic approaches have also been used to identify proteins that are abundant in medically important fungi, including Aspergillus, without having significant homology to human proteins.

Whole organism and other “crude” vaccines
Crude vaccines consisting of whole A. fumigatus (live or killed) or fractions derived from culture filtrates have been studied in mouse models of aspergillosis. Such studies provide a proof of principle. However, this type of approach may not reach human testing due to concerns about possible autoimmune responses and excess reactogenicity to the large numbers of antigens contained in such vaccines. Cenci et al. vaccinated mice by three intranasal inhalations with live A. fumigatus, heat-killed A. fumigatus, or a crude culture filtrate. They then immunosuppressed the mice with cyclophosphamide and challenged them with intranasal or intravenous A. fumigatus conidia. While survival was not prolonged in the mice that received heat-killed fungi, significant protection was observed in the mice that received live A. fumigatus or the crude filtrate. CD4+ T cells, IFN-γ, and IL-2 were required for protection. In contrast, mice that lacked IL-4 were more resistant to A. fumigatus challenge.

Subsequent studies demonstrated that immunization of mice with live (but not heat-killed) conidia induced IFN-γ-producing, Aspergillus-specific CD4+ T cell and humoral responses. In contrast, the heat-killed conidia induced CD4+ T cells that produced IL-4 and IL-13. The mechanism responsible for the disparate responses to heat-killed and live conidia remain speculative but could be related to some of the live conidia germinating into hyphae, which is the morphotype seen in invasive disease. Hyphae express a unique set of antigens and stimulate different pulmonary dendritic cell responses compared with conidia.
Subunit vaccines and monoclonal antibodies
A wide variety of Aspergillus subunit vaccines, defined as vaccines containing one or more purified components, has been tested in preclinical models. Recombinant Aspergillus proteins, which elicit vaccine-mediated protection in models of aspergillosis, include Asp f3, Gel1, Asp f9 (Crf1), Asp f16, and Pep1.25,26 The mechanism of protection has been presumed to be CD4+ T cells although in one study adoptive transfer of cytotoxic T lymphocytes specific for Asp f16 peptides extended survival in mice infected with A. fumigatus.27 Purified cell wall glycans also have been used as immunogens. Protection was observed following intranasal vaccination with α- and β-1,3-D-glucans but not with galactomannan.26 As T cells generally recognize peptides and not carbohydrates, it is likely that protection is antibody-mediated. Finally, mice vaccinated with cell wall mannans were protected from a challenge with A. fumigatus conidia, with enhanced protection seen if the mannan was conjugated to bovine serum albumin.28

Passive administration of a monoclonal antibody directed against cell surface A. fumigatus antigens extended the median survival of mice that received a lethal fungal challenge.29 In addition to raising the possibility that prophylactic administration of monoclonal antibodies could be given to high-risk patients, this study provides a proof of principle that vaccine-elicited antibodies can be protective. Although antibodies traditionally were thought to protect against mycoses by promoting opsonization and complement deposition, this antibody as well as another monoclonal antibody directed against cell wall catalase B had direct inhibitory activity against A. fumigatus.29,30

 Therapeutic vaccines
In patients who receive allogeneic hematopoietic transplants, most cases of invasive aspergillosis occur after neutrophil recovery, suggesting the importance of T-cell defenses, which are considerably slower to reconstitute. This creates a unique situation in which donor T cells can be expanded with antigens ex vivo and then adoptively transferred into the patient. In mouse studies, adoptive transfer of Aspergillus-specific CD4+ T cells extended the median survival of mice with invasive aspergillosis receiving allogeneic bone marrow transplant.31 This study provided the foundation for a study in humans with invasive aspergillosis following haploidentical hematopoietic transplantation.32 Donor CD4+ and IFN-γ-producing T cell clones specific for Aspergillus antigens were generated and expanded by incubating peripheral blood mononuclear cells with heat-killed conidia. Transplant recipients who developed evidence of invasive aspergillosis received adoptive T cells. Remarkably, 9 of 10 patients who received such immunotherapy resolved their infection. In contrast, resolution was seen in only 6 of 13 control patients who did not receive T cells.

A more rapid technique for T-cell expansion based on activation-dependent expression of CD154 and CD137 following incubation of peripheral blood mononuclear cells with recombinant A. fumigatus proteins was recently described although has yet to be tested on humans.33 Importantly, the generated T-cell lines showed high IFN-γ and IL-17 responses to fungal species from a wide range of medically important genera, including Aspergillus, Candida, Fusarium, Mucorales, and Scedosporium. The efficiency and efficacy of the process could be increased by the identification of additional antigens that stimulate human CD4+ T cells34,35 and by the simultaneous expansion of CD8+ T cell clones. Recently, a highly innovative technique to generate T cells reactive with Aspergillus and other fungal antigens was developed in which human T cells were genetically modified to express the β-glucan recognition receptor Dectin-1.36 Such bioengineered T cells avidly bound to and killed A. fumigatus germlings.

 Delivery systems and adjuvants
Purified antigens tend not to elicit strong immune responses unless they are administered with stimulatory adjuvants and/or delivery systems.8,37 For carbohydrate antigens such as β-1,3-D-glucon that are poorly immunogenic, conjugation with a protein carrier greatly boosts specific antibody responses.18 The adjuvant or delivery system used informs the nature of the immune response. Alum, the adjuvant used in most commercial vaccines, tends to bias toward antibody and Th2 cell responses. Bozza et al. were able to induce protective Th1-mediated responses using a vaccine consisting of dendritic cells pulsed with live A. fumigatus or A. fumigatus RNA.31 Another approach used dendritic cells transduced with an adenovirus vector encoding IL-12.38 Mice receiving DCs pulsed with heat-killed A. fumigatus had greater survival and lower fungal burdens.

Dendritic cell vaccines are probably too labor intensive and expensive for routine vaccination of a large population but they do have translational potential in therapeutic vaccines. Other studies successfully adjuvanted Aspergillus vaccines with unmethylated CpG-rich oligonucleotides (which are ligands for TLR9) and TiterMax.25,26,39 Interestingly, some candidate antigens, such as Asp f3 and Asp f9, have IgE binding epitopes and have been identified as allergens.7 However, when given with the proper adjuvant, they induce protective responses in mice.23 This raises the prospect that adjuvanted vaccines could be used in patients with allergic aspergillosis to convert deleterious atopic responses to ones that are more balanced.
Conclusions

Vaccination is arguably one of the greatest public health successes in the history of medicine. Nevertheless, effective fungal vaccines for clinical use have remained elusive; this despite the burgeoning numbers of persons with aspergillosis and other life-threatening fungal diseases. Advances in understanding the complex ways the immune system controls Aspergillus and what goes awry in immunocompromised populations have informed strategies for preventative vaccine development. Similarly, numerous immunogenic antigens have been identified at the molecular level, which could serve as candidate antigens in human vaccines. Novel adjuvants and delivery systems are becoming available that can skew vaccine responses toward those associated with protection. In a pilot trial, immunotherapy demonstrated great potential in allogenic hematopoietic transplant recipients with invasive aspergillosis; new technologies for expanding T cells promise to make this procedure safer and more efficacious. Despite the impressive progress, much work still needs to be done if vaccines against aspergillosis are to become a reality. Given the substantial morbidity and mortality associated with aspergillosis, it is truly a task worthy of hard study.

Acknowledgments

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Declaration of interest

The author reports no conflicts of interest. The author alone is responsible for the content and the writing of the paper.

References

6. Verweij PE, Chowdhary A, Melchers WJ et al. Azole resistance in Aspergillus fumigatus: Can we retain the clinical use of


