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COMMENTARY

Overcoming Hurdles to Development of a Vaccine against *Pneumocystis jirovecii*

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ABSTRACT Development of *Pneumocystis* pneumonia (PCP) is a common problem among immunosuppressed individuals. There are windows of opportunity in which vaccination would be beneficial, but to date, no vaccines have made it to clinical trials. Significant hurdles to vaccine development include host range specificity, making it difficult to translate from animal models to humans. Discovery of cross-reactive epitopes is critical to moving vaccine candidates from preclinical animal studies to clinical trials.

KEYWORDS *Pneumocystis carinii*, vaccines

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In this issue of *Infection and Immunity*, Tesini and colleagues report that immunization of mice with the N-terminal half of a protein termed *Pneumocystis* cross-reactive antigen 1 (Pca1) protected against *Pneumocystis* pneumonia (PCP) in a model of $CD4^+$ T cell depletion (1). They further found that antibodies produced in response to immunization in mice were able to recognize *Pneumocystis murina*, *Pneumocystis carinii*, and *Pneumocystis jirovecii*, the fungal species that infect mice, rats, and humans, respectively (1). This is the latest of a >30-year string of papers looking at immunization against *Pneumocystis* species using a number of animal models. However, this study is distinct as it demonstrates that a peptide cross-reactive with *P. jirovecii* is not only immunogenic but protective in immunosuppressed animals. The authors suggest that Pca1 could be used as a vaccine candidate in humans or as an immunogen for generating *Pneumocystis*-specific intravenous immunoglobulin (IVIG) for treatment of PCP.

Pneumocystis is an opportunistic fungal pathogen that was first identified as a parasite in the early 20th century. Pneumonia caused by *Pneumocystis* was first reported in premature or debilitated infants in Europe and was termed interstitial plasma cell pneumonia (2). Since then, PCP has been diagnosed in patients that have been immunocompromised as part of chemotherapy, transplant regimens, steroid treatment, and new biologics, including anti-tumor necrosis factor (anti-TNF) therapy for autoimmune diseases (3–6). PCP really came to prominence during the early days of the acquired immunodeficiency disease syndrome (AIDS) epidemic when it was one of the first indications that there was some sort of disease spreading that compromised the immune systems of previously healthy individuals (7). More recently, it has been appreciated that *Pneumocystis* is able to colonize the lungs, particularly in those who have chronic lung diseases, such as chronic obstructive pulmonary disease (COPD) (8). Though one might argue that development of a vaccine against an opportunistic pathogen is not cost-effective, modern medicine has led to many immunosuppressing agents that put patients at risk for PCP. These patients would benefit from an effective vaccine against *Pneumocystis*.

Over the past 35 years since the first reports describing PCP in AIDS patients, there have been a number of papers demonstrating that specific antibody can be protective against *Pneumocystis* infection. This is important since it is possible that circulating

antibody can be maintained long after CD4 T cells have been compromised. In fact, Gingo et al. found that elevated antibodies specific for the *P. jirovecii* kexin protein (KEX1) were associated with reduced risk for developing PCP in human immunodeficiency virus (HIV)-infected individuals (9). Most papers addressing immunization or vaccine development have utilized animal models of infection, including mice, rats, ferrets, and nonhuman primates. Early on, it was recognized that *Pneumocystis* species have host ranges such that organisms that infect one mammalian species do not cross over to other mammalian hosts. These species are now recognized as different species of *Pneumocystis*, with the species names *murina*, *carinii*, and *jirovecii* reserved for organisms that infect mice, rats, and humans, respectively. It was shown that immunization of mice with ferret-derived organisms was not protective against challenge with *P. murina*, indicating that organisms from other mammals cannot be used for human vaccines (10). Moreover, until recently, there have been few cross-reactive epitopes discovered between the *Pneumocystis* species. Though it has been shown that immunization with whole organisms isolated from a host can be protective in that same host species, using killed *P. jirovecii* as a vaccine is not tenable. There is no way to obtain sufficient *P. jirovecii* organisms, as no viable culture system is available to adequately expand the organisms for use in a commercial vaccine. This leaves us with finding a protective subunit vaccine which must be developed in animal models and then translated to humans.

The major surface glycoprotein (Msg, or glycoprotein A [gpA]) expressed by the organisms is encoded by a large multicopy gene family, with *P. jirovecii* having more than 3 times the copies that *P. murina* has. A large amount of diversity was found among the genes in the gpA gene family, and it is thought that this plays an important role in evasion of host defenses through antigenic variation (11). Vaccination against gpA in animal models has resulted in variable results, with some, but not complete, protection observed in a rat model of infection (12). In a mouse model of infection, it was shown that immunization with Msg resulted in an immune response but not protection against PCP (13). This is due to antigenic variation, since it was shown that passive immunization with monoclonal antibodies against one variant of gpA in severe combined immunodeficient (SCID) mice forced the emergence of organisms expressing a different gpA variant (14). A more recent paper indicated that immunization with recombinant variant Msg resulted in cross-reactive antibody responses to other Msg variants, but cross-reactive T cell responses were not frequently found, suggesting that Msg variation is used to escape T cell responses, rather than antibody responses (15). As a result of these studies, gpA has not been recently pursued as a viable vaccine candidate.

The search for viable vaccine candidates has evolved as more has been learned about *Pneumocystis* organisms. Several groups have reported that immunization of mice with sonicated or freeze-thawed *Pneumocystis* organisms resulted in IgA and IgG antibodies predominantly reactive to a 55- to 60-kDa antigen (16, 17). In one study, the antigen was loaded onto dendritic cells for vaccination, and the other study used antigen and cholera toxin B as an adjuvant, and both demonstrated protection from PCP when $CD4^+$ T cells were depleted from mice (16, 17). Several groups have examined the use of the *Pneumocystis* protease kexin (KEX1) as a potential vaccine target (18). Zheng et al. (2005) used DNA expressing the *P. murina* kexin and CD40 ligand (CD40L) in CD4 T cell-depleted mice to demonstrate that kexin-specific antibodies were produced corresponding to about a 100-fold reduction in *P. murina* lung burden (19). Interestingly, the antibodies recognized a 55-kDa protein on Western blots that was likely the same protein recognized by immunization with protein preparations from whole organisms (16, 17, 19). This group also demonstrated that antibodies raised to kexin in mice cross-reacted with *Pneumocystis* isolated from nonhuman primates (19). This past year, Kling and Norris demonstrated that immunization of nonhuman primates with KEX1 resulted in high titers of specific antibody and protection against PCP for almost 36 weeks after immunosuppression using infection with simian immunodeficiency virus (SIV) or HIV and exposure to *Pneumocystis* (20). Together, these

studies indicate that kexin has potential as a vaccine candidate. Direct demonstration of cross-reactivity with *P. jirovecii* has not yet been established for kexin.

Because *Pneumocystis* is an opportunistic pathogen, it is critical to demonstrate that vaccination results in protection during an immunosuppressed state. Animal models have been critical for this. Most models have utilized mice depleted of CD4 T cells using infusion of specific antibody. In these models, mice are vaccinated and then depleted of CD4 T cells prior to exposure to *Pneumocystis*. This is the approach taken by Tesini et al. (1), who demonstrated protection for 3 weeks and protective antibody titers for 9 weeks after immunosuppression. Further studies should be performed to demonstrate how long protection would last and whether vaccination would be effective in individuals with partially compromised CD4 T cell counts, as one might encounter with a patient infected with HIV treated with antiretroviral drugs. These studies are actually hard to perform in animal models, though nonhuman primates infected with SIV have proven to be a good option. There have been studies in which dendritic cells loaded with *Pneumocystis* antigen or DNA vaccines have been used in already CD4-depleted mice with positive results (17, 19). However, these types of approaches for infectious diseases, though promising, have not been approved for use in humans in the United States (21). For now, the most direct way to market is an adjuvanted vaccine delivered by injection or intranasally.

An important hurdle to developing vaccines to *Pneumocystis* has been the lack of a system for genetically manipulating the organisms. There is currently no reliable system for maintaining organisms in long-term culture, so understanding the genetics of *Pneumocystis* has been very difficult and slow going. Ruan et al. recently used a chemical labeling and proteomic approach to identify a putative surface protein from *P. murina* named SPD1 (22). Vaccination with the C-terminal portion of SPD1 resulted in antibody responses that corresponded with significant reduction in *Pneumocystis* lung burden in CD4 T cell-depleted mice (22). Serum antibody from SIV/HIV-infected rhesus macaques exposed to *Pneumocystis* recognized SPD1 (22). Identification of surface proteins using proteomic approaches will undoubtedly lead to identification of more vaccine candidates (23). The Gigliotti group took the approach some time ago of developing monoclonal antibodies against *Pneumocystis* organisms and using those antibodies for identifying surface epitopes that could be exploited for vaccination. Tesini et al. used a monoclonal antibody, 4F11, to identify the antigen Pca1 for use as an immunogen (1). This monoclonal antibody is one of only a couple that have been shown to cross-react with different *Pneumocystis* species, including *P. jirovecii*. A previously published paper demonstrated that the C-terminal portion of the protein, then called A12, was protective against PCP in CD4 T cell-depleted mice (24). The antiserum from those protected mice was able to recognize *Pneumocystis murina*, *P. carinii*, and *P. jirovecii*, making this a viable new vaccine target for PCP in humans.

CONCLUSIONS

There have been many hurdles to developing a vaccine against *Pneumocystis*. In addition to the question of whether a vaccine to an opportunistic pathogen would be profitable enough to entice pharmaceutical companies to invest in it, there are technical issues that make development very difficult. These issues include the *Pneumocystis* species barrier resulting in difficulty translating to humans, lack of a tractable genetic system, and inability to culture the organisms. All of these things make vaccine development slow going. Tesini et al. have made a leap forward by identifying a cross-reactive epitope that is protective in immunosuppressed mice depleted of CD4 T cells (1). Future work needs to determine whether the vaccine candidate is able to protect humans from PCP. With the publishing of the *P. jirovecii* genome and new approaches to recognize surface proteins, there may finally be a viable vaccine candidate that could soon leave the preclinical stage and go to clinical trials.

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