The Third NIAID Workshop in Medical Mycology: Immunology in Medical Mycology: Antigenic Peptides, Glycobiology and Vaccines

Feature Article

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Researchers Use Molecular Immunology and Technology to Combat Fungal Pathogens

The current focus on peptides and cell wall polysaccharides as candidate vaccines is part of a much broader NIAID vaccine development program

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Leading researchers studying a variety of fungal pathogens say that there is a major shift in thinking regarding vaccines. Thus, the prevailing question of whether vaccines should be considered as a practical way of preventing fungal diseases is being challenged by the questions of which ones and when. Key advances in research on several important fungal diseases, including histoplasmosis, coccidioido-mycosis, cryptococcosis, and even candidiasis, could soon bring vaccines for one or several of these diseases within reach. These basic advances are also leading to stepped-up efforts to establish the vital interdisciplinary framework needed within the research community if such valuable public health products are to be developed.

As part of this accelerated activity, the National Institute of Allergy and Infectious Diseases (NIAID) is sponsoring several workshops on medical mycology, the most recent of which was held 7-9 September 1995 in Big Sky, Mont. The Montana workshop brought investigators working on different fungal pathogens together with researchers from other fields, enabling the assembled group to better identify more of the many technological challenges that need to be overcome. Participants considered these challenges in terms of two distinct specialty areas of research: (i) antigenic peptides and proteins and (ii) glycobiology.

There is still much to be done before appropriate antigens can be isolated from fungal pathogens, characterized adequately, and administered in such a way that they elicit a safe and protective immune response in the host. For example, because standardization of strains and reagents is needed for these vaccine development efforts to succeed, a group of researchers is establishing a cryptococcal working group as one step toward achieving that interim requirement (see box, p. 82). In addition, a second workshop on fungal immunology is being planned for 1997.

The Importance for Vaccines of Fungal Peptides

Researchers are identifying a series of antigenic peptides from fungal pathogens that can generate immune responses, particularly cellular immune responses that are considered critical for protective immunity. As part of this response, T cells recognize fungal (or other foreign) peptides that bind to receptor molecules which are part of the major histocompatibility complex.

Fungal peptide vaccine candidates afford both advantages and disadvantages. On the plus side, peptides induce particular immune responses, including the induction of immunoprotective cytokines, the deployment of cytolytic T cells, and the production of antibodies. These peptides can be designed to delete suppressive epitopes that might prevent a useful immune system response.

CWG Forms

Because nonuniform strains of Cryptococcus neoformans are widely used to study pathogenesis and the host immune response, investigators are concerned that the results of their individual research efforts may not be generalizable. Since the number of investigators in the field is relatively small and the resources of individual laboratories are limited, a group of them decided during the NIAID Montana workshop to form the Cryptococcal Working Group (CWG), which is open to all interested individuals throughout the world.

The purpose of CWG is to identify areas in which scientists can establish collaborative efforts for resolving important questions of cryptococcus biology and pathogenesis. Since the Montana meeting, the Internet is proving a valuable means for CWG members to hold frequent informal discussions over the selection of representative cryptococcal strains for future studies. To expedite these efforts, Christopher Mody of the University of Calgary set up an electronic mail server for CWG. He can be reached by E-mail at cmody@acs.ucalgary.ca.

Arturo Casadevall

Arturo Casadevall is an assistant professor at Albert Einstein College of Medicine in Bronx, N.Y.

One of the difficulties in developing peptide-based vaccines is the poor immunogenicity of peptides when they are injected into animals. This problem can be partly overcome by inserting peptides into lipid complexes composed of palmitic acid. Furthermore, the immogenicity of certain peptides can be enhanced by linking them to helper epitopes.

Still other methods for producing peptide-based vaccines are being evaluated. For instance, researchers studying malaria have developed an experimental multiple-antigen peptide-based vaccine, and its efficacy is striking in experiments conducted with mice. This experimental malaria vaccine, which consists of linked multiple epitopes, thus may provide a model for the development of fungal vaccines.

Researchers are currently studying a number of peptides as potential vaccines against several fungal pathogens. Several examples were highlighted at the Montana workshop.

- Epitopes of a protective immunogen from *Histoplasma capsulatum,* namely, heat shock protein 60, are being mapped. In addition, considerable interest is focusing on the product of a yeast phase-specific gene from *H. capsulatum, yps3,* that is not detected in the mycelial phase. Although located in either the cell wall or cell membrane of the yeast, little is known about the function of this protein. However, because it stimulates human lymphocytes to proliferate, this protein is a target of interest to investigators interested in stimulating a protective immune response to this fungal pathogen.

- The gene coding for enolase from *Candida albicans* was recently cloned, sequenced, and expressed in a eukaryotic system. Although the recombinant

protein elicits a cellular immune response in animals colonized with this fungus, the protective efficacy of this immunogenic protein has not been evaluated.

- The gene of WI-1, a surface antigen from *Blastomyces dermatitidis*, contains multiple tandem repeats, an epidermal growth factor-like domain, and a region that has homology to invasin. WI-1 appears to be an adhesin molecule that mediates binding of this fungus to mononuclear phagocytes. In addition, it is a target of both cellular and humoral immune responses. Some of the T-cell reactive epitopes as well as the major histocompatibility complex restriction patterns of WI-1 have been identified.

- Two cloned and sequenced immunoreactive antigens from *Coccidioides immitis* are involved in the cellular immune response to this fungus. One antigen, which stimulates a T-cell line, is homologous to 4-hydroxy-phenylpyruvate dioxygenase and the mammalian F antigen; the other is heat shock protein 60. These antigens are being tested for their ability to protect mice against this pathogen.

A Potential Antigenic Role for Fungal Surface Polysaccharides

Virtually all medically important fungi carry an impressive array of polysaccharides on their cell surfaces, and these macromolecules play significant roles in fungus-host interactions. Yet despite more than 40 years of study of this class of fungal antigens, we still have little appreciation for their physical properties or their potential as vaccines.

"Antibody responses against carbohydrate epitopes typically involve only a limited number of (B-cell) clones, and the immunoglobulin subclass is often restricted," points out Tom Kozel of University of Nevada School of Medicine, Reno, who spoke during the workshop. This general phenomenon applies to at least some specific instances in which the immunogenicity of fungal polysaccharides has been evaluated. For example, the glucurono-xylomannan polysaccharide capsule of *Cryptococcus neoformans* stimulates antibodies directed against several epitopes, according to investigators in the laboratories of Kozel and Arturo Casadevall, who is at Albert Einstein College of Medicine in Bronx, N.Y. These antibodies vary widely in their protective efficacy. Efficacy also depends on the immunoglobulin isotype that is produced by the host defense against cryptococcosis, adds Casadevall.

For the fungi, the role of natural antibody immunity in protection is uncertain despite decades of investigation. However, the identification of protective monoclonal antibodies has raised hopes that vaccines that elicit antibody responses of the correct specificity and isotype for protective antibody immunity can be designed.

Specialized antibodies apparently can protect rodents against particular fungal pathogens, according to Casadevall and Jim Cutler of Montana State University, Bozeman, who is studying candidiasis. In addition to epitopes identified Casadevall within the capsular glucuronoxylomannan that seem to be protective against cryptococcosis, Cutler and his colleagues find that antibodies to an epitope within the acid-labile mannan portion of the phosphomannoprotein complex of *C. albicans* protect mice against disseminated candidiasis. These observations underscore the importance of defining precisely which fungal epitopes can induce protective responses by the host and then applying this information to the development of candidate vaccines.

Reinventing Fungal Vaccines

Current vaccine development efforts represent part of a long-term program aimed at preventing fungal infections. Now several new technologies and approaches are being brought to this challenge.

In the recent past, extensive work went into developing a vaccine for coccidioidomycosis. Those studies led to a phase III clinical trial involving nearly 3,000 volunteers (D. Pappagianis and the Valley Fever Vaccine Study Group, Am. Rev. Respir. Dis. 148:656-660, 1993). That clinical trial, conducted between 1980 and 1985, demonstrated a slight but statistically insignificant reduction in disease in the group vaccinated with formaldehyde-killed spherules of *Coccidioides immitis* compared to the unvaccinated control group.

Irritation at the injection site made it necessary to dilute the vaccine to 1/1,000 of the dose that prevented several species of animals from developing serious infections. According to the published report of that clinical study, "A different physical form other than the whole spherules must be sought to increase the tolerability of the immunogenic component."

Because the time seems right to adapt new methods to this purpose, the Rotary Americas Valley Fever Foundation recently launched a drive to raise money to help in developing and testing a vaccine for coccidioidomycosis. For more information about these fundraising efforts, contact Thomas Larwood, a physician in Bakersfield, Calif., at (805) 871-6090.

Technically, such approaches are at least feasible because it now is possible to determine very precisely the physical structure of fungal cell wall carbohydrate components. Several workshop participants, including Patrick Brennan of Colorado State University in Ft. Collins, Robert Cherniak of Georgia State University in Atlanta, and Roger O'Neill of Perkin-Elmer Applied Biosystem Division in Foster City, Calif., outlined recent developments that should enable researchers to map fungal component epitopes, including those contained within complex polysaccharides, that may induce protective antibodies.

Indeed, Brennan and his colleagues have developed a detailed model of the mycobacterial cell wall, combining molecular genetics techniques and sophisticated chemical instrumental analysis. This effort to define the cell wall structure of mycobacteria may well serve as an inspiration for investigators seeking a comparable understanding of the fungal cell wall. Cherniak, for instance, is following a similar approach in studying the cryptococcal capsular polysaccharide.

Insights from Other Efforts To Develop Vaccines

According to Milan Blake from the University of Iowa, there are three major phases in early vaccine development: (i) the discovery phase, in which important and perhaps critical immunogens are identified, purified, and subsequently tested in appropriate animal models; (ii) the transfer phase, in which the experimental vaccine is readied for clinical testing, standard production procedures are developed, and commercial development and licensing are considered; and (iii) the scale-up phase during which issues such as good manufacturing practices, the design and conduct of clinical trials for safety and efficacy, and questions of large-scale vaccine production and stability need to be systematically addressed.

Even with such general procedures in place for the development of vaccines, the details for any particular vaccine are important. Critically, small and seemingly trivial details can threaten a promising candidate vaccine, according to Blake. For instance, the development of a gonococcal vaccine based on the bacterium's porin molecule as the key antigen nearly failed in the early phase because of a trace contaminant, he says. Thus, the experimental vaccine contained miniscule amounts of Rmp (protein III), which induces anti-Rmp blocking antibodies that nullify

the protective effects of antiporin antibodies. The problem was resolved by genetically engineering a mutant source of porin that cannot produce Rmp because its gene is deleted.

The appropriate delivery of particular antigens, such as those derived from fungal pathogens, is another challenge for researchers developing vaccines, points out Roy Curtiss from Washington University in St. Louis, MO. He and his colleagues are developing recombinant avirulent bacterial systems as potential vectors for delivering a wide variety of vaccine components. For example, engineered versions of *Salmonella* spp. That constitutively express gene inserts induce both humoral and cellular immune responses when administered orally to rodents, he says. Moreover, recombinant *Salmonella* spp. with two or more attenuating mutations are avirulent but retain their tissue tropism.

Fungal Vaccine Studies Are Part of Larger Vaccine Program

Current efforts to develop vaccines against fungal pathogens are part of a far broader vaccine development program, according to John La Montagne, director of the Division of Microbiology and Infectious Diseases at NIAID. NIAID established its Program for the Accelerated Development of Vaccines in 1981, and its emphasis is on the major infectious diseases. Major achievements funded through that program include the development of vaccines for hepatitis B, invasive pneumococcal disease, pertussis, and *Haemophilus influenzae* type b meningitis. Considerable resources are now going toward development of genetically engineered vaccines.

At the Montana workshop, a number of key questions on the need and support for vaccine development in the fungal diseases were addressed and the current status of vaccine research in fungal diseases was summarized. Statistics on extramural funding in mycology by the National Institutes of Health (NIH) emphasized that mycology research applications have become highly competitive and the area continues to be enthusiastically supported by NIH.

Over the course of the workshop, it became clear that numerous members of the medical mycological, health science, and biotechnology communities could identify with the diversity of skills necessary to formulate and test the hypotheses related to the basic and applied aspects of protective immune responses to the medically important fungi. This understanding encourages a team approach for answering questions that could have broad applicability to public health.

Speakers:

- George S. Deepe, Jr., M.D., University of Cincinnati
- Alessandro Sette, Ph.D., Cytel Corporation
- James P. Tam, Ph.D., Vanderbilt University
- Paula R. Sundstrom, Ph.D., Ohio State University
- Bruce Klein, M.D., University of Wisconsin, Madison
- Theo N. Kirkland, M.D., VA Medical Center, San Diego
- Elizabeth J. Keath, Ph.D., St. Louis University
- Thomas R. Kozel, Ph.D. University of Nevada
- Patrick J. Brennan, Ph.D., Colorado State University
- Roger O'Neil, Ph.D., Perkin Elmer
- Jim E. Cutler, Ph.D., Montana State University
- Robert Cherniak, Ph.D., Georgia State University
- Arturo Casadevall, M.D., Ph.D., Albert Einstein College of Medicine
- Errol Reiss, Ph.D., Centers for Disease Control
- Roy Curtiss III, Ph.D., Washington University
- Milan S. Blake, Ph.D., Rockefeller University
- John R. La Montagne, Ph.D., National Institutes of Health

- Dennis M. Dixon, Ph.D., National Institutes of Health

Facilitators:

- Ward Bullock, M.D., University of Connecticut
- Judith Domer, Ph.D., Tulane Medical School
- Frank Gigliotti, M.D., University of Rochester
- Mary Lipscomb, M.D., University of New Mexico
- Mitchell Magee, Ph.D., Texas Center for Infectious Disease
- Martha Peck, The Burroughs Wellcome Fund