



## **SUMMARY OF THE NIAID EXPERT PANEL ON IMMUNITY AND BIODEFENSE**

**June 17, 2002**

**Bethesda Marriott Hotel  
Bethesda, Maryland**

### **INTRODUCTION**

The National Institute of Allergy and Infectious Diseases (NIAID) recently published a Strategic Plan for Biodefense Research (<http://www.niaid.nih.gov/dmid/pdf/strategic.pdf>) to address biomedical research needs in the areas of bioterrorism and emerging and re-emerging infectious diseases. For guidance in implementing this plan, the NIAID convened several expert panel meetings of scientific leaders to provide objective expertise and comprehensive advice.

*A Blue Ribbon Panel on Bioterrorism and Its Implications for Biomedical Research* met in February 2002 to outline a research agenda for CDC Category A agents of bioterrorism (see summary at <http://www.niaid.nih.gov/dmid/pdf/biotresearchagenda.pdf>).

*An Expert Panel on Atopic Dermatitis and Vaccinia Immunization* met in June 2002 to address the problems of morbidity and mortality associated with use of the current smallpox vaccine in atopic dermatitis patients.

*An Expert Panel on Immunity and Biodefense* was also convened by the NIAID in June 2002, to address the immunological aspects of biodefense preparedness research. A summary of this meeting is provided in the following pages. Panel members identified high priority research areas in immunology that would lead to improved biodefense strategies, and recommended methods by which these research goals might be achieved. The panel included internationally renowned immunologists from academia, industry, and the government with expertise in innate immunity, vaccine adjuvant biology, immune memory and vaccine development, immune epitope identification, and antibody and innate immune targets for therapy. The discussion focused on specific needs for research in these areas, as well as on logistical issues, such as research resource accessibility, industry-university-government collaborations, and increased training. Methods to facilitate the involvement of basic immunologists in biodefense research were also discussed, as well as methods to encourage immunologist-microbiologist-vaccinologist interactions, and to enhance the training of medical professionals in immunological research. A summary of panel recommendations is given below.

### **AREAS OF RESEARCH**

#### **Innate Immune Mechanisms**

Cells and soluble mediators of the innate immune system are constitutively present in the body. They directly provide the first lines of defense against invading pathogens, acting in an antigen nonspecific manner. They also induce costimulatory signals that help activate the adaptive immune responses of B and T lymphocytes to establish long term, antigen specific protection. Although continued basic research is needed to gain a more comprehensive understanding of innate immunity, recent progress in defining many of the mechanisms involved has already laid the groundwork to develop novel adjuvants for vaccines and novel approaches for short-term protection from infectious pathogens.

### Adjuvants for Vaccines

In addition to pathogen antigens, adjuvants are often needed to create efficacious vaccines. Adjuvants work in an antigen nonspecific manner by activating one or more component of the innate immune system. More basic information is needed on the cells that are affected by adjuvants and on the innate signaling pathways triggered in those cells. Methods are also needed to optimize adjuvanticity by targeting vaccine antigens to appropriate antigen presenting cell (APC) types, such as dendritic cells or macrophages; by targeting particular intracellular APC compartments for optimal antigen presentation to T cells; and by inducing appropriate APC maturation steps to optimize the stimulation of T cells, activate antibody production, and induce immune memory. Research should focus on the molecular mechanisms responsible for optimal antigen delivery and adjuvant activity in order to learn generalizable principles applicable to many vaccine candidates. For example, Fc or endocytic receptors should be tested as molecular routes for immunization, along with Toll-like and other families of innate receptors. The innate role of B cells in activating immunity also needs to be defined, beyond the well-known role of B cells as specific antibody producers. Furthermore, new innate target receptors should be discovered for adjuvant development, and be tested in animal systems with a variety of vaccine candidates.

Characterization of the innate immune responses to specific pathogens is also an important area of research, because little is known for most of the CDC/NIAID Category A-C pathogens. The particular type of innate response needed for initial protection, and the innate activity needed for translation into optimal adaptive immunity (e.g., cytotoxic T cells, antibodies, or specific antibody isotype) should be defined. In addition, detailed information on specific mechanisms used by certain pathogens to evade innate immunity may lead to safer or more effective vaccine formulations.

Adjuvants that target multiple innate immune receptors may prove to be the most effective. More sophisticated knowledge of the microbial components that trigger innate responses should lead to more potent and precise adjuvants that have fewer harmful side effects. Such components include lipids, glycolipids, complex glycans, and peptidoglycans, as well as proteins. Given recent discoveries that inhibitory as well as stimulatory immune responses are induced in the vaccine context, it is important to develop vaccines that minimize inhibitory responses in a manner consistent with safe vaccine delivery to the civilian population.

It is important to understand the innate immune status and inflammatory cascades present at different stages following infection by specific pathogens. Understanding APC function and regulation *in vivo* is also an important and challenging area for new research, and better experimental systems that can define responses early in the process of innate and adaptive immune activation are needed. Furthermore, the probable need to treat individuals after exposure to a bioterrorist agent means that a better understanding of interventions that enhance or modify an ongoing innate or adaptive response will be of great value. Studies of chronic infectious disease might provide a useful starting point for such research and might describe common pathways for intervention in a number of different infections.

### Short-Term Protection

Emerging evidence strongly indicates that short-term, antigen-nonspecific protection against infection can be induced by triggering innate receptors even in the absence of specific antigen. This stand-alone approach could be especially valuable for biodefense when the specific pathogen is not known. However, recent evidence for both direct and indirect desensitization of innate receptor pathways after initial stimulation suggests that the timing of stand-alone innate-based treatments may be critical, to avoid increased rather than decreased susceptibility to infection.

Many infectious diseases involve a common cast of innate mediators, some of which contribute significantly to pathogenesis. For example, TNF $\alpha$  and IL-1 $\beta$  are likely to mediate immune injury during inflammation and septicemia. Such damage might be minimized or negated using agents that are already FDA-approved for other indications. In addition, common signaling pathways may be used by innate mediators, and such biochemical pathways might be targeted by drugs already in development, such as those that inhibit MAP kinases. Many of the Category A agents appear similar at certain stages of pathogenesis. For example, there is rapid onset of disease and sepsis-like disease at terminal stages. Therefore, common approaches might be effective at boosting early protective immunity or at limiting later immune-mediated damage.

Certain mediators of innate immunity, such as defensins and mannose-binding proteins, can directly target pathogens for destruction and may prove useful as broadly reactive biodefense tools, even when the pathogen is unknown. They might be used for short-term pre-exposure prophylaxis, for post-exposure treatment, or for short-term protection of populations unresponsive to or adversely affected by specific vaccines or stand-alone adjuvants. This area of research is just beginning to bear fruit, and would benefit from an expanded effort in the context of bioterrorist agents or emerging/re-emerging infectious diseases.

### **Adaptive Immune Mechanisms**

#### T and B Effector Cells

The rapidity of inducing protective T cell or antibody responses is especially important in the context of biodefense, since most of the population will not be pre-immunized. Sophisticated immunological studies are needed to define effective responses to existing

vaccines that rapidly induce protective immunity, such as the live vaccinia vaccine for smallpox, in comparison with much less potent vaccines, such as the current anthrax vaccine. Studies of immunological events that occur in the early stages of response are likely to be the most informative for vaccine development or improvement. The molecular mechanisms that control robust early protection by T cells or antibody are not completely understood, and the most effective type of response will vary depending on the individual pathogen. Large scale genomic and proteomic studies may be of particular use in this context, to help define response patterns that can be dissected to determine the key molecular events.

Although available for some Category A-C pathogens, early immune correlates of protection must be developed for many others. Animal models will be useful in this regard, and models that most closely resemble the human response must be developed. In some cases, immunized humans can be studied for immune parameters to help define useful surrogate markers. Clearly, sponsors of vaccine trials should make every effort to include sophisticated immunological research studies, in addition to endpoint studies, in conjunction with vaccine trials. Furthermore, basic immunologists should identify such trials and establish collaborations to conduct accompanying studies to define immunological mechanisms of protection.

The panel strongly recommended that centralized repositories of tissue samples and patient data from vaccine trials be established and made accessible to immunological investigators whenever possible. Not all studies need be done in the context of Category A-C agents; highly valuable information can be obtained from studies on currently licensed vaccines against other pathogens as well.

#### T and B Cell Memory

The longevity of a protective response elicited by a vaccine is also important when considering its prophylactic use to protect the group of first responders likely to be exposed in an attack. Again, little is known about immune memory, especially in the human, and considerable basic research is needed to identify the most effective memory cell subsets and their mechanisms of regulation. Reliable markers of human memory cells are needed, the basis for immunodominant antigen presentation should be defined, and new assays for evaluating T and B cell memory status should be developed. In addition, the mechanisms responsible for effective “boosting” by secondary immunization should be determined to discover whether common pathways may be targeted in different vaccines.

#### Epitope Identification

Epitope mapping is useful to identify the appropriate pathogen antigen for use in subunit vaccines. In addition, T and B cell epitopes can serve as specific immunogen sequences for vaccines or as targets for antibody therapy. Very few epitopes have been described for Category A-C pathogens. In general, the identification of protective T and B cell epitopes is a complex process, especially for CD4 T cell epitopes recognized in the context of MHC class II molecules. Algorithms that utilize pathogen genomic sequences to predict peptides that bind to particular MHC molecules are often correct, although vast

improvements could be made if the number of known epitopes were increased. However, recent data indicate that there is not a one-to-one correspondence between the affinity of a peptide for a MHC molecule and its representation on the APC surface. Thus, MHC binding ability is only the beginning of the story. Antigen processing pathways in APC must be able to generate the predicted peptide in sufficient quantities for presentation to T cells, and the ability of the MHC-peptide complex to stimulate T cells must be assessed. Finally, the peptide must be tested *in vivo* to evaluate its probable utility as a protective vaccine immunogen. The phenomenon of peptide immunodominance must also be addressed and is currently poorly understood. Therefore, many basic questions remain to be answered.

MHC-binding peptides can now be identified with outstanding sensitivity using mass spectrometric analysis of peptides eluted from isolated MHC molecules. Thus, panels of peptide candidates that are processed and bound to MHC are easily obtained in many cases. But the cellular source of the MHC molecules must still be verified as relevant to the induction of protective T cell responses, and appropriate T cell activation by the MHC-peptide complex must be shown. In addition to peptides, some T cells recognize specific lipids or carbohydrates. The characterization of such epitopes is still rudimentary and basic research is clearly needed in this area.

Like T cell epitopes, antibody epitopes are not well defined for Category A-C pathogens. These epitopes are recognized by the B cell antigen receptor (BCR) to stimulate antibody production, and are recognized by soluble antibodies that clear pathogens from the body. Therefore, the identification of antibody epitopes will facilitate both vaccine development and the development of passive immunotherapeutic agents. Predictive algorithms based solely on gene sequence are not very successful, and most predictive methods also depend on structural information to assess the likely accessibility of a candidate epitope to an antibody or BCR. Therefore, increased involvement of structural chemists in vaccine or immunotherapeutic design is strongly encouraged. Because pathogens may have evolved to evade antibody-mediated destruction, clever new approaches may be needed to define protective, rather than useless or even enhancing, epitopes for antibodies.

### **Human Immunology**

A strong recommendation was made to facilitate human immunological research in the context of biodefense. In particular, greater access is needed to human tissues and relevant patient information, requiring considerable coordination of immunologists with clinicians and clinical departments. Human immunology workshops, perhaps modeled on the current CD antigen workshops, would facilitate the characterization, standardization, and exchange of necessary reagents. In addition, centralized repositories, and formal research collaborations between immunologists and vaccinologists conducting clinical trials, were recommended to help overcome the considerable roadblocks that currently exist for basic immunologists who want to move from animal model systems into human research. Appropriate support for research nurses and administrative personnel is also needed to facilitate immunology research in humans. It was recommended that relevant

NIAID programs, such as the Vaccine Treatment and Evaluation Units, be made accessible to basic immunologists to conduct mechanistic studies that accompany vaccine trials.

### **Immunity for Infants, the Elderly, and the Immunocompromised**

The immunological status of the human population is highly variable. Clearly, normal as well as premature infants, young children, pregnant women, the elderly, and those who are immunocompromised due to underlying disease, chemotherapy treatment, or immunosuppression after allotransplantation are at greater risk of some infections and are less likely to respond favorably to some vaccines. Little is known about the fundamental differences known to exist between the healthy adult immune system and the immune system in early development or in senescence. Improved technologies are now available to begin more comprehensive immunological studies of these populations in normal situations, as well as in the context of vaccine trials. Interference by maternal antibodies in the vaccination of infants is an important area of study, as is the potential “imprinting” of immune response types in babies exposed to infections or vaccines *in utero*. In the elderly, the use of multiple medications targeting multiple physiological systems may impact the effectiveness of certain vaccines. Patients with primary or secondary immunodeficiencies will need protection *via* passive immunotherapies for diseases not yet susceptible to drug treatments. Meaningful immunological studies in these special populations will require the considerable involvement of clinicians as well as basic immunologists, and infrastructure to support such studies should be expanded or created where necessary, and made accessible to researchers.

### **RESEARCH RESOURCE NEEDS**

The panel strongly recommended that core resource facilities be established to provide reagents, tissue samples, standardized *in vitro* and *in vivo* experimental systems, Biosafety Level 3/4 laboratories, Good Manufacturing Practice and Good Laboratory Practice grade products, and scientific expertise to all relevant biodefense researchers. The Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases Research will at least partially serve this purpose, together with a planned NIAID Research Resource Repository; an animal models program for *in vivo* testing for licensure requirements, including a nonhuman primate program; and Cooperative Centers for Translational Research on Human Immunology and Biodefense. Thus, current plans include the support of resources that will be available to both basic and applied researchers, but may require expansion to meet the growing need.

New infrastructure is also needed to overcome the bottleneck in moving a product from the laboratory into Phase I clinical trials. One particular opportunity for NIH leadership is the testing of new adjuvant candidates. In this regard, lessons applicable to infectious agents might be learned from the study of adjuvants currently being tested in cancer vaccines.

Centralized programs for comprehensive, large-scale, systematic genomic and proteomic analyses of immune responses to infection or vaccination were also recommended by the panel. Such programs would generate essential data sets made freely available to the research community.

## **INDUSTRY INVOLVEMENT**

Clearly, pharmaceutical and biotechnology companies can play major roles in the biodefense research effort, providing basic research resources as well as conducting product development programs. Many companies are able to work with outside researchers, especially if the development of products applicable to many diseases, such as new adjuvants, might be leveraged by collaboration with academic or government groups. NIAID programs such as the Partnerships for Biodefense will facilitate industry involvement and enable participation by many commercial collaborators critically important to the research and development effort. It is already evident that there is considerable commercial interest in the development of new adjuvants, vaccines, immunotherapeutic reagents, and diagnostic devices.

## **RESEARCH TRAINING PROGRAMS**

Many different types of scientific and clinical expertise are needed for implementation of a successful and enduring biodefense research program. Clearly, better communication and formal collaborations among immunologists, microbiologists, and vaccinologists will be fostered by multi-project research initiatives and centers. However, many more clinical researchers are needed, and incentives to train specialists in pediatric and geriatric infectious disease and immunology research would be especially valuable. NIH mechanisms to support both clinical and basic research training are currently in place, and will be expanded under the biodefense program. Existing training programs should expand to support the immunological training of both PhD and MD students and postdoctoral fellows, and new programs that emphasize multidisciplinary training focused on the immunity of infection should be established. If possible, loan forgiveness programs should be enlarged and targeted to MD scientists engaged in biodefense research.

## **ROLE FOR THE IMMUNOLOGY COMMUNITY**

The wealth of knowledge on immunity and immune regulation produced over the past two decades provides an exceptional foundation for continued advances in both basic and clinical research critical for biodefense. Many productive scientists currently studying basic immunological processes and principles can and will contribute greatly to the biodefense effort by incorporating studies on immune responses to Category A-C pathogens into their research programs, or by expanding current basic studies of innate immune mechanisms, immune recognition, or immune memory. Other immunologists should be recruited into this effort through collaborations fostered by multidisciplinary projects, and by accessibility to research resources.

Abundant expertise currently exists and can be developed to ensure that basic immunology research both benefits from increased opportunities under new biodefense programs and contributes to the rapid development of new vaccines, immunotherapeutic treatments, and diagnostic products needed to protect against bioterrorist agents and emerging and re-emerging infectious diseases.

**National Institute of Allergy and Infectious Diseases  
Expert Panel on Immunity and Biodefense  
June 17, 2002**

**Bethesda Marriott Hotel  
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