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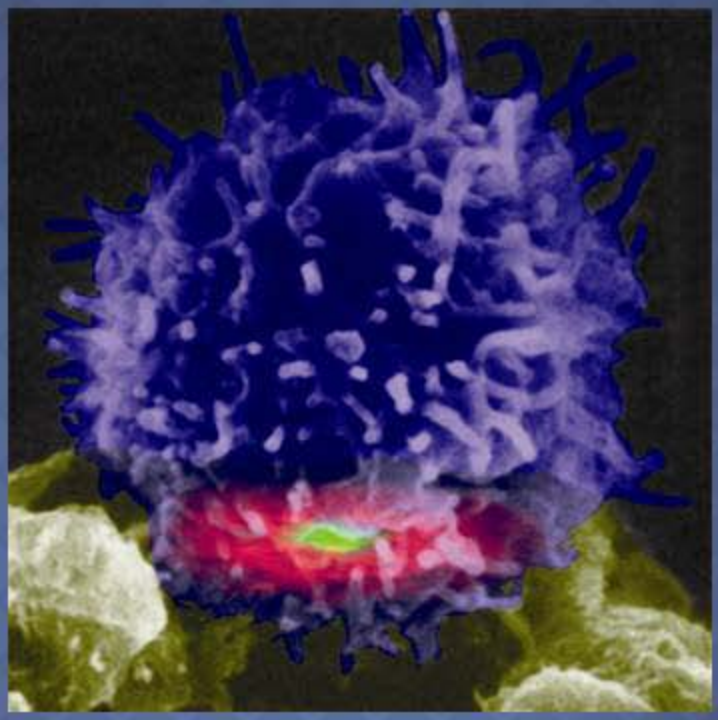
National Institute of Allergy and Infectious Diseases

NIAID STRATEGIC PLAN FOR RESEARCH ON VACCINE ADJUVANTS



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National Institute of Allergy and Infectious Diseases

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NIAID STRATEGIC PLAN FOR RESEARCH ON VACCINE ADJUVANTS

INTRODUCTION

Vaccines to prevent infectious diseases are the most effective and economical measure to improve human health and have saved millions of lives worldwide. The use of vaccines has led to the elimination of smallpox, the near eradication of polio, and protection against seasonal influenza and a large number of childhood diseases. Yet, there remain many devastating infectious diseases for which no effective preventive vaccines exist, including diseases of great consequence to global health, such as malaria, tuberculosis, and HIV/AIDS; as well as diseases caused by newly emerging infections. Increasingly, efforts to develop efficacious vaccines against these and other infectious diseases involve the use of adjuvants in vaccine formulations. The U.S. Food and Drug Administration (FDA) defines adjuvants as "agents added to, or used in conjunction with, vaccine antigens to augment or potentiate (and possibly target) the specific immune response to the antigen." Adjuvants have the potential to make current vaccines more effective, and to make vaccines more readily available to larger numbers of people worldwide. Additionally, adjuvants have the potential to help create vaccines against pathogens for which no vaccines currently exist, and could be especially important for vaccines made from recombinant antigens or DNA, which tend to be poorly immunogenic and do not generally elicit a protective response when used alone. Moreover, given that special populations, such as the elderly, young children, and immunocompromised individuals, often respond suboptimally to vaccination, the addition of an appropriate adjuvant to a vaccine may enhance the limited immune responses in these groups. Adjuvants can also extend the supply of a vaccine by allowing dose-sparing of the vaccine antigen or reducing the number of immunizations required to generate and maintain protective immune responses. As researchers develop a better understanding of the mechanism of action of different adjuvants, the rational design of adjuvants will result in adjuvanted vaccines that can induce the most effective immune responses against specific pathogens.

The National Institute of Allergy and Infectious Diseases (NIAID) has developed a Strategic Plan for Research on Vaccine Adjuvants to guide its adjuvant discovery, development, and translational research program. This strategic plan summarizes the current status of NIAID-sponsored research in the field of adjuvants for preventive vaccines, identifies gaps in knowledge and capabilities, and defines NIAID's goals for the continued discovery, development, and application of adjuvants for human vaccines that protect against infectious diseases. Both preventive and therapeutic vaccines are of importance to human health. The strategic plan focuses only on adjuvants for use with preventive vaccines that block infection. Therapeutic vaccines, those to be administered only after established infection, and cancer vaccines, may require scientific approaches and clinical development plans that differ from those for preventive vaccines. Appendix 1 outlines the planning process used to develop the draft strategic plan.

The NIAID Strategic Plan for Research on Vaccine Adjuvants is divided into three sections: (1) Basic Immunology and Early Stage Adjuvant Discovery; (2) Later Stage Adjuvant Development and Preclinical Testing; and (3) Clinical Assessment of Adjuvants. This strategic plan is intended to strengthen and guide the NIAID adjuvant research enterprise, accelerate the

pathway from adjuvant discovery to U.S. licensure of adjuvanted vaccines, and promote increased adjuvant research and development partnerships among Government, academia, non-profit institutions, and industry. In implementing this strategic plan, NIAID will continue to work closely with its partners in the research community on adjuvant discovery and development within available resources and the context of its overall strategic research mission.

Scientific Background

Adjuvants

The term adjuvant is derived from the Latin word *adjuvare*, which means to help. It applies to compounds that target the innate immune system and enhance immune responses to co-administered vaccines. Normally, the first line of defense against microbial infections is the activation of the innate immune system; this leads to the activation of T and B cells of the adaptive immune system to generate antigen specific responses resulting in pathogen clearance. Adjuvanted vaccines can accelerate the production of protective antibodies and effector T cell responses and prolong the duration of protection by expanding memory B and T cell populations. Most licensed vaccines are thought to mediate protection by antibodies. Adjuvants such as alum (the term used for several insoluble aluminum salts, usually aluminum phosphate or hydroxide), MF59, and monophosphoryl lipid A (MPL) enhance antibody production, but are limited in their ability to induce T cell immunity. By enhancing adaptive immune responses, adjuvants may reduce the number of required immunizations or the amount of antigen needed to elicit a protective immune response; furthermore, they might allow for more durable protection. In addition to this quantitative effect, vaccine adjuvants may also provide qualitative benefits in immune responses, such as broader cross-protection to related, heterologous strains of pathogens. By exploiting these effects on the strength and diversity of adaptive immune responses, adjuvants may prove to be critical for the design of vaccines of increased immunogenicity and efficacy in special populations. For example, an MF59-adjuvanted influenza vaccine demonstrated enhanced immunogenicity in children and the elderly and greater clinical efficacy in elderly nursing-home residents. Recent limitations in the vaccine supply for pandemic influenza strains also highlight the potential for dose-sparing that can be achieved by adjuvanted vaccines intended for large populations.

Despite the benefits that adjuvants confer, the molecular mechanisms underlying their activity are only partially understood. Adjuvants are believed to activate the innate immune system, at least in part, by targeting professional antigen presenting cells, such as dendritic cells, leading to the upregulation of cytokines, costimulatory molecules, and MHC molecules resulting in the migration and recruitment of effector cells and the activation of antigen specific T cells. Novel insights for rational development of new adjuvants arose in the 1990s from the discovery and characterization of Toll-like receptors (TLRs). TLRs are proteins that belong to a growing number of Pattern Recognition Receptor (PRR) families and they are expressed on the cell surface or within the cytosol of innate immune cells. Cell surface and intracellular TLRs and other PRRs recognize Pathogen Associated Molecular Patterns (PAMPs), which are products of bacteria, viruses, protozoa, and fungi. Upon recognition of PAMPs, the TLRs initiate a signaling cascade leading to release of cytokines and chemokines, maturation of antigen presenting cells and immune activation. Other recently characterized PRRs recognize microbial derivatives in

the cytosol, and include the nucleotide-binding oligomerization domain-like receptor (NLR) family of proteins recently identified as components of the inflammasome; the RNA-detecting retinoic acid inducible gene-based-I-like helicase receptor family (RIG-I, MDA5); and at least two DNA-sensors, the DNA-dependent activator of interferon-regulatory factors (DAI) and the molecule "absent in melanoma 2" (AIM2). Other types of non-TLR PRRs include Dectin-1, a natural killer (NK) cell receptor-like C-type lectin involved in innate immune responses to fungal pathogens; complement components; and scavenger receptors expressed by macrophages and dendritic cells, which play an important role in uptake and clearance of microbes and their products.

Vaccines made from inactivated or live-attenuated pathogens generally contain naturally occurring molecules such as PAMPs that act as adjuvants. In contrast, vaccines made from recombinant proteins or purified subunits of a pathogen are often poorly immunogenic because they lack the endogenous innate immunostimulatory components of the pathogen and, therefore, may necessitate the addition of an adjuvant to elicit an effective immune response. Some polysaccharide conjugate vaccines, such as Prevnar-7 and -13, are formulated with alum as an adjuvant, although others are effective in the absence of any known adjuvant; it is not yet known whether the latter vaccines engage innate immune pathways.

Previously, vaccines and adjuvants were developed empirically, but an emerging understanding of innate immune receptors and signaling pathways is now providing opportunities for the rational design of novel adjuvants. More recently, knowledge of many components of the innate immune system has led to a new understanding of the mechanism of action of alum, the most widely used adjuvant in human vaccines. Alum was the first vaccine adjuvant to be widely used in the U.S. beginning in the 1920s. It was thought initially to function primarily by its ability to adsorb vaccine antigens, enabling the antigen-alum complex to function as a depot that allows the gradual release of antigen over time. More current work, published in 2008 by several groups, demonstrated that activation of the NALP3 inflammasome also plays a role in alum adjuvanticity; however, it is still unclear if alum acts directly on the inflammasome or indirectly through other innate immune mediators.

A "one size fits all" adjuvant will not be ideal for all vaccines because qualitatively distinct responses may be needed in certain settings. Adjuvanted vaccines that use alum result in a predominant Th2 response and antibody production. In contrast, Th1 responses or cytotoxic $CD8^+$ T cell responses may be required to generate protective immunity to certain pathogens. Thus, there are numerous efforts to develop different adjuvants that can direct immune responses to activate different T cell subsets, to target particular antigen presenting cell subsets, or to target mucosal responses.

Besides alum-containing vaccines, no other adjuvanted vaccines received approval by the U.S. Food and Drug Administration until 2009, when the human papilloma virus vaccine Cervarix containing AS04 was approved. AS04 contains both alum and MPL, which is a component of lipopolysaccharide (LPS) and is the first TLR ligand approved as a vaccine adjuvant in humans worldwide. AS04 was also approved in Europe as a component of Fendrix, a hepatitis B virus vaccine. From the mid-1990s to the present, other adjuvants have gained regulatory approval in Europe, including MF59 and liposomes for inclusion in influenza vaccines (Fluad and Inflexal,

respectively). Also in Europe, AS03, a squalene-based adjuvant, was used in the 2009 H1N1 pandemic influenza vaccine Pandemrix. Thus, a number of new adjuvanted vaccines have recently been licensed and others are being evaluated in clinical trials (see Appendix 2).

Based on their dominant mechanisms of action, the few approved and many experimental adjuvants have frequently been divided into two classes: immunopotentiators or antigen delivery systems; however, there appears to be some functional overlap between the classes. A number of newly discovered adjuvants target TLRs or NLRs, such as MPL (TLR4) and muramyl dipeptide (NOD2), CpG oligonucleotides (TLR9), flagellin (TLR5), and single or double stranded RNAs (TLR3, 7, or 8). The hepatitis B vaccine, human papilloma virus vaccine, and malaria RTS,S candidate vaccine are all particle formulations. Particulate adjuvants such as liposomes, virosomes, ISCOMs (immune stimulatory complexes), nanoemulsions, or virus like particles are used to encapsulate and enhance delivery of antigen, and some may have additional immunostimulatory properties. In addition, cytokines and chemokines can be broadly included as adjuvants when incorporated into DNA vaccines. Other classes of adjuvants include emulsions (MF59 or Montanide ISA-51), saponins (QS21), bacterial toxins (cholera toxin), polysaccharides (Advax, a crystalline particle derived from inulin), and cell based adjuvants (e.g., antigen-pulsed dendritic cells). Understanding how effective vaccines work has led to testing combinations of adjuvants, as in AS04, to target multiple receptors or pathways mimicking natural infection. Many other natural and synthetic compounds have been shown to have adjuvant activity and currently are being studied.

Governmental Sponsorship of Research on Adjuvant Discovery and Development

In addition to the pharmaceutical and biotechnology industries, a number of non-governmental organizations and governmental agencies fund research on adjuvant discovery and development. Among the U.S. Federal agencies, major support is provided by the Department of Defense (Defense Advanced Research Projects Agency, Walter Reed Army Institute of Research, and the Defense Threat Reduction Agency) and the Department of Health and Human Services (NIAID, the National Cancer Institute, the National Institute of Child Health and Human Development, Centers for Disease Control and Prevention [CDC], FDA, and the Biomedical Advanced Research and Development Authority [BARDA]). After the events of September 11, 2001, and the anthrax mailings just a little later that year, considerable resources and efforts have been directed at the development of countermeasures related to biological agents that pose the greatest threat to civilian populations, including naturally emerging pathogens such as the SARS, West Nile, dengue, and pandemic influenza viruses (http://www3.niaid.nih.gov/Biodefense/bandc_priority.htm). Several NIAID planning documents (http://www.niaid.nih.gov/topics/biodefenserelated/biodefense/about/pages/strategicplan.aspx) provided an early impetus for development of NIAID's current adjuvant research programs, but did not define a set of research goals and recommendations focused specifically on adjuvants.

Federal agencies, including NIAID, also collaborate on adjuvant discovery and development with global partners, including the World Health Organization (WHO) and the European Medicines Agency (EMA). A number of workshops and meetings were convened and guidelines

published to assess regulatory issues regarding the use of adjuvants in humans. Examples of these activities include:

- A December 2002 meeting of the FDA Center for Biologics Evaluation and Research and the Society of Toxicology focused on the nonclinical safety evaluation of vaccines
- WHO guidelines published in 2003, including the nonclinical evaluation of vaccines, with a section on adjuvants
- A 2005 EMA publication of guidelines on adjuvants in vaccines for human use
- A December 2008 joint workshop, sponsored by the FDA and NIAID, on adjuvants and adjuvanted preventive and therapeutic vaccines for infectious diseases

In addition, the European Adjuvant Advisory Committee (EAAC), comprised mostly of biotechnology and pharmaceutical companies, was established in 2003 to promote consensus on issues related to adjuvant development and use, as well as to encourage further adjuvant research in Europe.

To make adjuvants more accessible to the broader research community for testing in experimental vaccines, the WHO coordinates the Global Adjuvant Development Initiative (GADI). GADI was created to support the evaluation and comparison of different adjuvants through a network of laboratories called AdjuNet, which facilitates access to a variety of adjuvants for new vaccine development. In 2009, the European Network of Vaccine Research and Development (TRANSVAC) was established. Through TRANSVAC, thirteen European institutions in five countries share resources for vaccine development. In collaboration with this network, the Platform for Harmonization of Vaccine Adjuvant Testing (PHARVAT) will choose assays to test adjuvants for future use in vaccines. It is clear that adjuvant discovery and development is a global enterprise that will benefit from greater harmonization and wider access to certain reagents and research resources including animal models.

Evolution of NIAID Adjuvant Research Programs

Within the National Institutes of Health (NIH), NIAID is the lead Institute for comprehensive research on infectious diseases. NIAID's research mission includes understanding the biology of infectious agents and the pathogenesis of infectious diseases, the response of the host to infection or vaccination, and the basic immunological principles of protection against infection. Key to this effort is support for development of diagnostics, therapeutics, and vaccines to prevent infectious diseases. Basic, translational, and clinical research on adjuvant discovery and development play a critical role in this endeavor.

Through multiple programs, NIAID supports the development of new candidate vaccine adjuvants that stimulate the innate immune response to initiate a protective vaccine response against infection. Previous NIAID Research Agendas on Category A-C Priority Pathogens and an Expert Panel on Immunity and Biodefense (http://www.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/Documents/biosp2007.pdf) have pointed to the need for novel adjuvants and identified this as a gap in our knowledge. Such adjuvants would have the potential to improve current vaccines, to serve as essential components of novel vaccines against diseases for which effective vaccines are currently lacking, and to extend vaccine supplies to larger numbers of people worldwide. The 2007 update of the NIAID

Strategic Plan for Biodefense Research focused on new approaches that have implications broader than one reagent for one pathogen. The goal was to establish a more flexible and broad spectrum approach, including the development of "universal" vaccines. Development and utilization of a range of new adjuvant platforms may be one means to achieve this.

The NIAID Strategic Plan for Biodefense Research recommends the evaluation of inducers of innate immunity for use as first-line therapies and as adjuvants for augmenting vaccine efficacy against pathogens that might be used in a bioterrorist attack. In an effort to promote the discovery of additional immune stimulating molecules, the NIAID Division of Allergy, Immunology and Transplantation (DAIT) established the Innate Immune Receptors and Adjuvant Discovery Program in 2003. This program was renewed in 2009, to continue support for identification and optimization of lead candidates for adjuvant discovery.

In 2008, to move beyond the discovery phase and begin development of lead adjuvant candidates, DAIT initiated a new Adjuvant Development Program to advance novel vaccine adjuvants towards licensure for human use. This initiative supports the advancement of candidate vaccine adjuvants through immunological characterization studies, lead compound optimization, and/or Investigational New Drug (IND)-enabling studies. Also in 2008, research on innate immune receptors was further supported by the establishment of a DAIT program for Reagent Development for Toll-like and Other Innate Immune Receptors. This program, funded through a cooperative agreement, supports the development of new reagents and research tools to study the expression and function of TLRs and other innate immune receptors in humans and animal model systems. These programs that are specifically focused on adjuvants are complemented by many additional projects within broader NIAID research programs and as individual investigator-initiated grants (for a more detailed summary, see Appendix 3). Despite the increase in adjuvant research in recent years, many gaps still exist in our basic understanding of, and practical experience with, adjuvants for preventive vaccines. The following sections address these gaps and outline NIAID goals to advance progress in adjuvanted vaccine development.

NIAID STRATEGIC PLAN FOR RESEARCH ON VACCINE ADJUVANTS

I. Basic Immunology and Early Stage Adjuvant Discovery

Understanding host protective immune responses to pathogens is critical for developing preventive vaccines. NIAID currently supports a wide range of research projects that explore the basic immunology of the innate immune system and its interactions with, and induction of, adaptive immune responses. This work includes studies on the cells and receptors that are targets of adjuvant activity and signaling pathways that modulate adjuvant activity. In addition, many current studies focus on understanding the development of T and B cell memory responses. Other recent efforts seek to identify immune correlates of protection that are induced during infection or following successful immunization. Building on this foundation, many of the current adjuvant candidates were identified after basic discoveries in immunology pointed to new receptors, pathways, and cells as possible targets for development of novel adjuvants.

Recent insights into innate immunity also provide a foundation for the development of new vaccine adjuvants that are tailored to stimulate particular immune responses or that might be optimal for particular pathogens. Initial work in adjuvant discovery was focused on compounds that activate various TLRs, and potential adjuvant targets have expanded with new discoveries in innate immunity. Despite an abundance of early stage candidates, only limited attention has been paid to combinations of adjuvants. This will certainly be a fruitful area for future research, because simultaneous targeting of distinct innate immune pathways has the potential to increase vaccine immunogenicity and efficacy while avoiding harmful side effects.

High throughput screening allows researchers to test large molecular libraries for compounds with previously unknown adjuvant activity, or that generate unique responses or signal through specific molecular pathways. Genomic and proteomic approaches have led to deeper insights regarding signaling components of innate immune pathways, which are potential adjuvant targets. These techniques generate large and complex data sets, and sophisticated computational analyses will be required to make optimal use of such information. Eventually, such approaches will provide a more comprehensive view of the affected subcellular signaling pathways and point to targets for further development. Further development includes the application of medicinal chemistry approaches such as structure-activity relationships (SAR) to rationally modify an adjuvant candidate. Important parameters that can be manipulated by structural modifications include receptor binding specificity and affinity, differential pathway triggering, and reduction of off-target effects. With better ability to manipulate the pathways at the interface of innate and adaptive immunity, researchers will be able to focus on adjuvant targets that stimulate potent responses while minimizing undesirable effects.

Immediate Goals:

- Maintain a robust portfolio of basic research on innate immune receptors and their natural ligands in animal and human cells
- Identify local innate immune responses to adjuvant:vaccine administration and the optimal routes of administration that lead to durable protection at specific mucosal sites
- Determine adjuvant effects on inducing antibody isotypes and T cell subsets that provide mucosal protection
- Establish a compendium of human gene expression patterns and other biomarkers that correlate with adjuvant activities
- Understand the mechanisms of action of known and novel adjuvants, and combinations of adjuvants
- Apply Structure-Activity Relationship approaches to address adjuvant specificity, potency, and toxicity
- Discover or engineer adjuvants that potentiate specific types of immune responses; for example, the induction of cellular responses versus the generation of antibodies, or Th1 versus Th2 type immunity
- Early in the adjuvant discovery phase, determine how formulation influences adjuvant activity
- Study cellular targets of adjuvants in addition to dendritic cells, such as mast cells, natural killer T cells, and γδ T cells

- Develop additional systems immunology programs and support focused training programs to optimize the use of genomics, transcriptomics, and bioinformatics to map signaling pathways relevant to adjuvant responses
- Promote collaborative multidisciplinary work by teams of immunologists, microbiologists, virologists, mycologists, parasitologists, and vaccine development experts including formulation scientists

Long-Term Goals:

- Understand the immunological rules that determine the activities of different classes of adjuvants
- Develop a balanced pipeline of adjuvants with the capability to:
    - induce qualitatively distinct types of immune responses for rational design of vaccines against a wide range of pathogens
    - target immune responses in neonates, the elderly, and immunosuppressed populations
- Develop widely applicable strategies to enhance immunogenicity while minimizing reactogenicity
- Study veterinary vaccines to compare adjvuants and determine immune mechanisms of action
- Investigate how microbial flora influence immune responses to novel adjuvants

II. Later Stage Adjuvant Development and Preclinical Testing

Later stage development of candidate adjuvants must proceed through immunological studies, lead compound optimization and formulation, stability testing, pharmacodynamic and pharmacokinetic determinations, and toxicology studies. Pre-clinical testing and evaluation of adjuvant lead compounds will also include safety studies in appropriate animal models, *in vivo* localization and clearance studies, dose assessments, and determination of activity via different routes of administration.

In vivo testing of the adjuvant with the vaccine antigen is essential. Species differences in innate immune receptor expression and associated pathways will require the development of strategies to assess *in vivo* the immunogenicity of adjuvant and antigen combinations. Dose ranging of the adjuvant, the antigen, and combinations of the two, and determining optimal routes of administration in animal models can provide valuable information to begin to estimate appropriate doses, formulations, and routes of administration for human use. Data on the specific immune response, such as gene expression, cytokine or chemokine patterns, correlated with safety and efficacy data, should provide valuable information for predicting immunogenicity in subsequent clinical trials. Thus, the preclinical studies should provide a rational basis for the design of clinical trials using promising adjuvant candidates.

Certain natural products have been identified as adjuvant candidates, but are often chemically heterogeneous and methods for their uniform production and quality control are also needed. In addition, manufacture of vaccines and adjuvants must be done according to current Good Manufacturing Practices (cGMP) to ensure the purity and consistency of product lots. It should

be noted that many of these activities are beyond the reach of academic laboratories and small biotechnology companies.

Immediate Goals:

- Advance promising adjuvant candidates through optimization and preclinical testing stages
- Foster collaborations between basic immunologists, adjuvant researchers, and formulation experts to determine the effects of formulation on the immunogenicity, efficacy, and safety of adjuvanted vaccines
- Assess the potential of combinations of adjuvants for particular vaccines and define methods to analyze mechanisms of action different from those conferred by each adjuvant alone
- Explore the usefulness of various adjuvants in enhancing the efficacy of vaccines delivered in heterologous prime-boost immunization regimens
- Promote collaborations among investigators to test adjuvant candidates with multiple vaccine antigens in a variety of experimental systems
- Expand access to animal model resources including humanized mice, nonhuman primates, and neonatal and aged animal models
- Provide animal model adjuvant testing services
- Support a centralized repository to distribute experimental adjuvants, model antigens (vaccines), and/or formulated standardized vaccines to the research community to test in different vaccine systems, while preserving intellectual property rights of the developer

Long-Term Goals:

- Test adjuvant:vaccine combinations that selectively promote Th1, Th2, Th17, or Treg, or that selectively target and preferentially activate specific subpopulations of antigen presenting cells (dendritic cells, B cells, monocytes/macrophages)
- Improve and standardize animal models for adjuvant testing of safety and efficacy; expand understanding of the strengths and limitations of particular model systems
- Develop novel antibodies and other reagents for use in a variety of non-murine animal models
- Determine optimal routes of administration, including mucosal administration, to produce the most relevant immune response against different classes of pathogens
- Develop public-private partnerships to help translate fundamental findings of novel adjuvants to later stage development, including product manufacture
- Develop standardized *in vitro* tests of adjuvant activities that correlate with *in vivo* outcomes
- Develop methods to evaluate the potential for long-term adverse reactions to an adjuvanted vaccine in preclinical models

III. Clinical Assessment of Adjuvants

The NIAID Division of Intramural Research (DIR), Division of Acquired Immunodeficiency Syndrome (DAIDS), the Vaccine Research Center (VRC), and Division of Microbiology and Infectious Diseases (DMID) all support clinical trials to improve current vaccines and to develop new vaccines for infectious diseases. DAIDS and DMID have established clinical trial networks

that could implement future studies on adjuvants for HIV-1 vaccines or vaccines against opportunistic infections, as well as other infectious diseases. DAIT supports targeted research programs that study the responses to infection or vaccination of special populations, such as the elderly, young children, and immunocompromised individuals. The purpose of these programs is to identify human immune response parameters and defects that differ from the effective responses to infection and vaccination seen in healthy adults. In addition, genomic profiling and the discovery of human SNPs (single nucleotide polymorphisms) that might help predict the outcome of vaccination or infection are being studied in different populations.

NIAID supports clinical trials for the development of vaccines against specific pathogens and some of these trials include adjuvants as vaccine components. NIAID has worked closely with colleagues in academia, industry, and the FDA to support trials that are well planned and efficiently executed. NIAID will build on past experience in this area to help ensure the successful design, implementation, and completion of future trials to evaluate novel adjuvanted vaccines and develop robust adjuvant:vaccine formulations appropriate for use even in resource-poor areas.

NIAID has a long and fruitful record of collaboration with industry and private foundations in carrying out vaccine clinical trials, which will continue to be of great value in studies to assess new adjuvants.

Immediate Goals:

- Collaborate with industry sponsors in phase I clinical trials of promising adjuvanted vaccine candidates to assess toxicity and reactogenicity using 1:1 randomization
- Utilize current NIAID Vaccine Treatment and Evaluation Units and other clinical trial networks to conduct adjuvant:vaccine trials and associated ancillary studies to assess immune mechanisms of action
- Promote interaction with NIH-supported human immunology programs
- Ensure that appropriate control arms are included in NIAID-sponsored adjuvant:vaccine clinical trials
- Optimize clinical trial design to improve statistical power and provide more definitive outcome results
- Identify candidate biomarkers that correlate with genetics, immunogenicity and reactogenicity
- Develop sample sparing assays, multiplex assays, and other methods to optimize the use of blood and tissue samples collected during clinical trials
- Standardize and optimize assays to rapidly and accurately assess immunogenicity, safety, and efficacy
- Develop new and improve current bioinformatics approaches to analyze clinical trial data
- Harmonize definitions for efficacy and adverse events/reactions
- Facilitate early interactions of investigators and NIAID program managers with regulatory agencies

Long-Term Goals:

- Collaborate with industry sponsors in phase II clinical trials of current and new vaccine candidates that incorporate novel adjuvants
- Correlate immune response measurements with individual parameters such as age, ethnicity, genotype, gender, and underlying chronic illness
- Use correlates of immunogenicity obtained from clinical trials to develop *in vitro* assays to predict toxicity and reactogenicity of future adjuvant:vaccine combinations
- Develop novel assays to assess mucosal protection in humans
- Establish biomarkers for safety and efficacy and test their predictive value
- Work with CDC, FDA, and industry to design appropriate clinical studies to generate and evaluate data for long-term safety evaluation
- Work with BARDA and other government agencies to support the development of non-commercial but necessary adjuvanted vaccines through phase III clinical trials

Appendix 1: Planning Process

The assessment and planning process for this strategic plan included the following activities:

- Review current adjuvant-related research and development activities supported by NIAID
- Define the current scope of adjuvant discovery and development in the broader research community
- Identify key stakeholders and partnerships, academic research organizations, U.S. Government agencies, not-for-profit organizations, vaccine and pharmaceutical companies
- Identify areas to be considered:
    - Current understanding of adjuvant discovery and development research, and mechanistic studies of adjuvants
    - Gaps in current knowledge of adjuvant mechanisms of action
    - Opportunities for scientific advancement based on greater understanding of innate immunity
    - Barriers to adjuvant discovery and development
    - Resources and partnerships required for advancement
    - Future areas for research and development activities for adjuvants
- Identify future areas for initiative development within NIAID to provide support for novel adjuvant approaches

Based on this assessment, immediate and long-term goals were identified for NIAID in three major areas:

- Basic Immunology and Early Stage Adjuvant Discovery
- Later Stage Adjuvant Development and Preclinical Testing
- Clinical Assessment of Adjuvants

Appendix 2: Examples of Adjuvant Types Approved for Human Use in Preventive Vaccines or Tested in Human Studies

Category	Examples	Target (likely)	Approved
Aluminum salts	Alum	(NALP3)	Multiple vaccines; *worldwide*
Pathogen components	PamCSK	TLR2	No
	PolyI:C	TLR3	No
	MPL/TLR-4 agonists	TLR4	*EU only*
	Flagellin	TLR5	No
	R848, Gardiquimod	TLR7/8	No
	CpG DNA	TLR9	No
	Muramyl-dipeptide	NOD2	No
Emulsions	Freund's adjuvant	(APC)	No
	MF59	(APC)	Fluad (influenza); *EU only*
	Montanide	(APC)	No
Nanoemulsions	NB-series	(TLR)	No
Liposomes	Virosomes	APC	Inflexal (influenza), Epaxal (HAV); *EU only*
Microparticles	PLG	APC	No
	ISCOM	(APC)	No
Cytokines	IL-1, IL-2, IL-12	Cytokine receptor	No
	GM-CSF	Cytokine receptor	No
Oligosaccharides	Inulin-derivative	Macrophage/ phagocytosis	No
Combinations	AS0-series	Multiple	Pandemrix (influenza); Fendrix (HBV); *EU only* Cervarix (HPV); *EU/U.S.*
	IC31	Multiple (including TLR9)	No

Appendix 3: Status of NIAID Adjuvant Research Activities

INTRODUCTION

NIAID is the major NIH Institute supporting research in the area of vaccine adjuvants, funding 65-70% of all extramural projects on adjuvants, with another 20% funded by the National Cancer Institute and the remainder funded across 11 other Institutes. Within the NIAID intramural program, approximately 10 projects involve adjuvant research. Among approximately 140 extramural projects, 55 are funded as unsolicited investigator-initiated grants, and 85 as solicited cooperative agreement grants or contracts. Across NIAID, a small number of training grants focus on adjuvant discovery and mechanisms of action. Similarly, a small number of NIAID small business innovative research (SBIR) and small business technology transfer research (STTR) grants focus on this area, with topics that include adjuvants for mucosal vaccine delivery, synthetic ligands for innate immune receptors, and development of virus-like particles (VLP) that incorporate adjuvants together with vaccine antigens.

Described below are selected examples of NIAID-supported research on adjuvants to enhance or help create new preventive vaccines against infectious disease. The work is organized according to the various Divisions in NIAID and includes research ranging from the discovery of novel adjuvant compounds to the testing of adjuvanted vaccine candidates in humans.

NIAID EXTRAMURAL RESEARCH ACTIVITIES

Division of Allergy, Immunology and Transplantation (DAIT)

DAIT is the lead extramural NIAID Division for the discovery and characterization of vaccine adjuvants, and it supports a portfolio of adjuvant research projects focused on the following research topics:

- Basic research on innate immune receptors and their ligands to identify new adjuvant targets
- Discovery of novel adjuvant candidates and platforms using high throughput screening approaches
- Early development of vaccine adjuvants
- Immunological basis of adjuvants in current vaccines
- Mucosal immunology
- Research resources

Basic research on innate immune receptors and their ligands to identify new adjuvant targets. DAIT supports the basic immunology of individual candidate adjuvants to assess their potential to enhance vaccine efficacy without inducing toxicity. Most of this work is supported by investigator-initiated grants, and several projects are funded under a DAIT-solicited research program, the Cooperative Centers for Translational Research on Human Immunology. Topics include:

- Structural studies of innate immune receptors
- Direct targeting of human dendritic cells using antigen conjugated to antibodies that bind dendritic cell surface proteins

- Defective interfering Sendai virus genome particles as novel adjuvants
- Sulfoglucosylceramide to induce NKT cell responses and promote activation of dendritic cells for antigen presentation
- Non-propagating Venezuelan equine encephalitis virus replicon particles, which target to draining lymph nodes and induce mucosal immunity
- Complement receptor agonists, such as C3d, to enhance antibody responses to vaccine antigens

Discovery of novel adjuvant candidates and platforms using high throughput screening approaches. In 2003 and 2004, as part of its biodefense research program, DAIT solicited projects under a new Innate Immune Receptor and Adjuvant Discovery Program, and funded five contracts to discover novel adjuvants based on interactions with TLRs. These projects used high throughput methods to screen small molecule and natural product libraries to identify novel compounds with adjuvant activity. Each contract resulted in the identification of one or more lead compounds for further development. In 2009, this program was renewed to support the discovery of additional lead compounds, expanding the scope to innate immune receptor targets in addition to TLR, through six new contracts. Topics include:

- Small molecule screens of chemical libraries to identify agonists for RIG-I receptors, NOD-like receptors, and TLRs
- Nanoemulsion formulations that activate innate immune responses without signs of inflammation

Early development of vaccine adjuvants. In 2008 and 2009, DAIT funded four contracts for the further development of novel adjuvant candidates, beyond the discovery stage, under its new Adjuvant Development Program. This program supports the optimization of lead adjuvant candidates, formulation studies, and preclinical pharmacology, toxicity, and efficacy studies. Topics include:

- CpG oligodeoxynucleotides as TLR9 agonists
- Glucopyranosyl lipid A and R848 as TLR agonists
- Delta-inulin, a natural plant polysaccharide, to activate signal transduction pathways
- Aminoalkyl glucosaminide phosphate as a TLR4 agonist
- Synthetic, chemically defined versions of naturally occurring adjuvant compounds
- Structural variants and "minimal congeners" of known adjuvants that retain immunostimulatory properties of the parent molecule, but have minimal reactogenicity
- Small molecule analogs of known adjuvants with improved pharmacological properties

Immunological basis of adjuvants in current vaccines. DAIT-funded work in this area includes mechanistic studies of known adjuvants in order to link their *in vivo* properties to specific immunological parameters, such as identification of their cellular and molecular targets, the signaling pathways induced, and the quality of the adaptive B and T cell immune responses that are enhanced, including antibody isotypes, cytotoxic T cell activities, and involvement of chemotactic factors and dendritic cells, macrophages, mast cells, NK cells, NKT cells, and γδT cells. Research also includes the characterization of intrinsic adjuvant activity present in live-attenuated, inactivated virion, and carbohydrate vaccines. While much of this work is supported by investigator-initiated grants, efforts in this area will receive enhanced support through the

recently-awarded Human Immunology Project Consortium program that will include studies of human immune responses to current vaccines. Topics include:

- Mechanisms of action of alum and oil-in-water emulsion adjuvants
- Properties of biopolymers acting as both emulsifiers and immunomodulators
- Molecular basis of adjuvant activation of innate immune responses
- Pathways associated with adjuvant pyrogenicity and reactogenicity
- Molecular signatures of adjuvants in human vaccinees

Mucosal immunology. In 2011, DAIT will expand its targeted research program by establishing the Immune Defense at the Mucosa Cooperative Study Group, which will support additional research in this area. Future NIAID activities will build on this research foundation.

Research resources. DAIT supports a cooperative agreement grants program on Reagent Development for Toll-like and Other Innate Immune Receptors, focused on generating new reagents for the analysis and modification of innate immune responses. Four groups are funded to discover, characterize, and distribute reagents that are broadly useful to the scientific community for research on vaccine adjuvants. Topics include:

- High throughput screens of peptide mimetic chemical libraries to identify novel TLR4 agonists and antagonists
- High throughput screens of small molecule libraries to identify novel agonists and antagonists of TLR3, TLR7, and RIG-I
- Production of antibodies and soluble receptors reactive with innate immune cell scavenger receptors
- Identification and characterization of novel members of NOD-like receptor families and the generation of antibodies reactive with each new protein

Division of Microbiology and Infectious Diseases (DMID)

DMID is the lead extramural NIAID Division for the development of vaccines against non-HIV infectious agents, including preclinical and clinical testing of adjuvants to define new preventive adjuvant:vaccine formulations. DMID supports a portfolio of adjuvant research projects focused on improving current vaccines and creating vaccines for infectious agents that have been intractable to prior vaccine development efforts. This work is supported by investigator-initiated grants as well as cooperative agreement grants under the Regional Centers of Excellence for Biodefense and Emerging Infectious Diseases Program and a series of Partnership programs funding collaborative research between academic and commercial institutions.

These projects include the following types of research:

- Basic research to discover effective combinations of pathogen antigens with known adjuvants
- Targeted development of new adjuvant:vaccine candidates
- Clinical testing of new adjuvant:vaccine candidates
- Research resources

Basic research to discover effective combinations of pathogen antigens with known adjuvants. DMID funds a comprehensive array of basic science projects on vaccines for a variety of bacteria, viruses, parasites, and fungi, to identify and characterize the most effective

combination of known adjuvants with pathogen antigens for the prevention or treatment of infection, or to allow antigen dose sparing. Topics include:

- Optimization of TLR4 ligands as adjuvants for *Yersinia pestis* vaccine development
- Use of CpG, monophosphoryl lipid A, Glucopyranosyl Lipid A (GLA), and other TLR agonists in various vehicles or combinations (e.g., oil-in-water emulsion, aqueous formulation, liposome, and alum) as adjuvants for Leishmania vaccine development
- Heat shock proteins as adjuvants for protective cytotoxic T cells against influenza
- Development of novel schistosome vaccines employing the Sm-p80 schistosome antigen-based DNA construct with Th1 cytokines or using the recombinant protein-based immunogen in combination with alum, TLR4,7, 8, or 9 agonists
- *Mycobacterium tuberculosis* epitopes incorporated into virus vectors together with one or more known adjuvants
- Incorporation of the QS21 adjuvant into alum-adjuvanted anthrax vaccines
- Replication-restricted vesicular stomatitis virus as a vector to generate immunity to *Francisella tularensis* in combination with IL-12 or GM-CSF
- Alum, CpG, and GLA adjuvants used for novel vaccines against *Entamoeba histolytica*

Targeted development of new adjuvant:vaccine candidates. DMID also supports the discovery of novel adjuvants for use in the development of pathogen-specific vaccines. Topics include:

- Mucosal adjuvants based on the edema toxin of *Bacillus anthracis*
- Co-adjuvant activity of NAD (nicotinamide adenine dinucleotide), which targets the cellular receptor CD38, to promote leukocyte migration to sites of influenza infection
- A powder vaccine formulation for nasal immunization against West Nile Virus using small molecule mast cell activating compounds as adjuvants, either alone or in combination with TLR agonists
- Novel cationic lipid DNA complexes to enhance immune responses against influenza, *Burkholderia mallei*, and *Burkholderia pseudomallei*
- Novel lipid A compounds based on low toxicity lipopolysaccharides isolated from anaerobic gram negative bacteria as adjuvants in experimental vaccines against *Yersinia pestis* and *Francisella tularensis*
- Self-assembling polypeptide nanoparticles, and a VLP carrier system composed of the woodchuck hepadnavirus core protein, as adjuvants for malaria vaccines that genetically display B and T cell epitopes of *Plasmodium falciparum*
- A human hepatitis B core antigen-based VLP for chemical conjugation with *Plasmodium vivax* proteins
- Novel, inulin-based microparticles that stimulate adaptive immune responses to influenza and other vaccines, with dose sparing effects

Clinical testing of new adjuvant:vaccine candidates. DMID supports clinical trials of new adjuvant:vaccine combinations through its clinical networks such as the Vaccine Treatment and Evaluation Units.

Research resources. In 2003, as part of its biodefense program, DMID established the Biodefense and Emerging Infections (BEI) Research Resources Repository, which provides a

broad spectrum of research resources such as pathogens, plasmids, proteins and peptides; cell lines; and antibodies reactive with a variety of immune molecules, including receptors and ligands of the innate immune system that are useful in adjuvant studies (www.beiresources.org). DMID also supports development of animal models and provides access to these models for researchers to evaluate novel vaccine candidates.

Division of Acquired Immune Deficiency Syndrome (DAIDS)

DAIDS research includes a portfolio of grants that study adjuvants in the context of HIV-1 vaccine development. The discovery of novel adjuvant candidates is supported partly through the DAIDS Center for HIV/AIDS Vaccine Immunology Program, which is exploring the use of certain polymers and chemoattractant cytokines as enhancing components for HIV-1 vaccines.

Other adjuvant work is supported by unsolicited investigator-initiated and solicited cooperative agreement grants, with a focus on using known adjuvants to help generate protective anti-HIV-1 or -SIV immunity. Topics include:

- Mechanisms of QS21 activity, to design improved analogs with fewer safety issues
- Preclinical studies of novel HIV-1 structures and epitopes as immunogens combined with known adjuvants
- Plasmid forms of mucosally expressed chemokines such as CTACK, MEC, and TECK as adjuvants with HIV-1 DNA vaccines
- HIV-1 VLP administered together with C3d or CpG
- GM-CSF adjuvant activity with DNA prime/MVA boost strategies for HIV-1 vaccine development
- IL-12 and IL-15 as adjuvants for HIV-1 vaccine development
- siRNA as an adjuvant approach to inhibit negative regulators of immune activation for HIV-1 vaccine development

NIAID INTRAMURAL RESEARCH ACTIVITIES

Division of Intramural Research (DIR)

DIR researchers are studying the basic biology of infectious diseases and mechanisms of immunity to pathogens, and are also conducting translational work for new vaccine development.

The Laboratory of Malaria Immunology and Vaccinology, formerly the Malaria Vaccine Development Branch, incorporates adjuvant studies into its general vaccine development path. The primary goal is to enhance immunogenicity of malaria vaccine candidates. Over the years, the laboratory evaluated vaccine candidates formulated with various adjuvants including alum, Montanide, MF59, ISCOMs, QS21, liposomes, and multiple TLR agonists in preclinical animal studies. The laboratory also demonstrated that the immunogenicity and response longevity of malaria antigens may be enhanced by conjugation to carrier proteins such as the outer membrane protein complex of *Neisseria meningitidis* and a non-toxic ExoProtein A (EPA) of *Pseudomonas aeruginosa*. These carrier proteins served as adjuvants. The laboratory is also forming new partnerships to evaluate novel adjuvants.

The adjuvanticity of CpG 7909 for a candidate vaccine against clinical malaria is being tested in U.S. and Malian adult volunteers. Alum and GLA were shown to further enhance the

immunogenicity of a Pfs25-EPA conjugate, a malaria transmission-blocking vaccine candidate. A phase I trial is being planned to evaluate safety and immunogenicity of the Pfs25-EPA conjugate formulated with Alhydrogel or with GLA.

The Laboratory of Infectious Diseases has used adjuvants for respiratory virus vaccines in preclinical studies. Addition of the adjuvants AS01(B) or AS03 to an inactivated SARS-CoV vaccine resulted in enhanced antibody titers and prolonged protection in rodents. Addition of a stabilized chemical analog of double-stranded RNA (PIKA) as an adjuvant to an inactivated influenza H5N1 influenza virus vaccine resulted in antigen sparing and both quantitative and qualitative improvements of the immune responses in mice.

The Laboratory of Immunology is engaged in studies examining the distribution of adjuvants and adjuvanted vaccine antigens, their effects on the behavior of immune cells *in vivo*, and the functional consequences of these effects using advanced imaging tools and other platforms. The Program in Systems Immunology and Infectious Disease Modeling is using high throughput RNAi screening to better understand signaling through PRRs such as TLRs and to develop computational models of PRR signaling pathways that can be used to help predict the effects of single and combination adjuvant agents. Through its involvement in the Trans-NIH Center for Human Immunology, the NIAID DIR is conducting studies aimed at developing an extensive database of the state of the normal immune system to serve as a basis for comparison of measurements made in volunteers and during clinical trials of adjuvants and adjuvanted vaccines. Currently, studies of influenza vaccines are underway, and future plans include studies on the role of adjuvants in the efficacy of hepatitis B vaccines, as well as immune signatures of such adjuvants as MF59.

Vaccine Research Center (VRC)

The primary focus of the VRC is to develop, produce, and test vaccines against HIV-1 and a variety of other human pathogens. In the context of HIV-1 vaccine development, the VRC is conducting pre-clinical studies on the incorporation of known adjuvants into vectored vaccine candidates using adenovirus, lymphocytic choriomeningitis virus, and Bacillus Calmette-Guerin (BCG) vectors for antigen delivery. In addition, candidate vaccines based on soluble HIV-1 env trimers with heterologous trimerization motifs are being tested in macaques with or without co-administration of the GSK adjuvant, AS01B, a mixture of monophosphoryl lipid A and QS21.

Protein-based adjuvants and vaccines are being tested in combination with other vaccine modalities such as viral vectors, DNA, and BCG, using prime-boost strategies. In particular, prime-boost regimens are under study as highly promising methods to induce optimal T cell- and antibody-mediated immunity for a variety of human pathogens.

Specifically, novel prime-boost approaches are being tested to create a “universal” influenza vaccine that could provide broad protection against diverse influenza virus strains. Recent results indicate that a variety of H1N1 strains isolated over the past 70 years could be neutralized after immunization of mice or ferrets with a plasmid HA-DNA prime/inactivated seasonal influenza vaccine boost, or a plasmid HA-DNA prime/HA-adenovirus 5 (replication-defective) boost regimen. Interestingly, many of the antibodies generated were reactive with the conserved stem region of the HA molecule. In addition to neutralization of diverse H1 viruses, some cross-

neutralization was also achieved for H3 and H5 viruses. These results suggest that new generation influenza vaccines that provide extensive heterosubtypic immunity might be feasible.