VACCINES: Entering a Bold New Era

Tuesday 16 September 2014

Programme organized by
Professor Stanley A Plotkin and
Professor Adel AF Mahmoud
Schedule
TUESDAY 16 SEPTEMBER

Programme organized by Stanley Plotkin and Adel Mahmoud

12:00 noon  Welcome
             Victor Dzau

12:05pm     Stanley Plotkin
             A short history of vaccines from Edward Jenner to the present day

12:15pm     Barney Graham
             Advances in structural biology and structure-guided vaccine design

12:25pm     David Weiner
             DNA vaccines, mRNA vaccines and novel delivery mechanisms (electroporation)

12:35pm     Gary Nabel
             Progress in the development of a universal influenza vaccine

12:45pm     Alejandro Balazs
             A novel vaccine approach: Programming an immune response that stops HIV transmission

12:55pm     Late Breaker
             Update on Ebola vaccine progress

1:00pm      Robert Tjian
             Closing remarks

1:05pm      Open floor discussion
             Moderated by Victor Dzau and Robert Tjian with Stanley Plotkin and Adel Mahmoud

1:30pm      End of briefing

This event complies with Senate ethics rules.
The benefits of investing in vaccine research and development

We stand poised on the threshold of an exciting new era in vaccine research and development. Recent advances in science and technology hold the promise that we can develop vaccines for many of the world’s most intractable diseases and infections, even cancer. During this briefing organized by the Foundation for Vaccine Research in partnership with the Institute of Medicine you will learn how these advances can lead to new or improved vaccines for many of the 39 major diseases and infections that have so far resisted our efforts to develop vaccines because they were so hard to crack and the science was not there.

Vaccines are miracles that have saved millions of lives and have a remarkable record of safety. New vaccines can save millions more lives and reduce suffering around the world. Supporting these science advances and ensuring that scientists have the resources they need to build on them may be our greatest challenge at a time when resources are scarce. Investing in vaccine research now can save billions of dollars in treatment costs later. The social and economic return on investment is extraordinarily high. Vaccines are the most cost-effective public health intervention since clean water. For just a few dollars per child, they can prevent disease and disability for a lifetime. They can reduce the burden of disease and relieve the stress on health care systems and the toll on health care workers. Yet vaccine research has been chronically underfunded. Of the billions invested in global health each year using the tools we have, less than two percent goes to vaccine research.1

Each year, more than three million people still die from HIV, malaria and TB, alone. Vaccines will one day be able to prevent these diseases and other infections, such as dengue fever, cytomegalovirus, hepatitis C and E, herpes, RSV, staphylococcus and streptococcal infections. Vaccines can prevent the outbreak of new and emerging diseases, such as MERS and Ebola. If given the support that’s needed, the dream of a universal flu vaccine that would dispense with the need for an annual flu shot could become a reality in our lifetime.

In the future, one can imagine vaccines controlling chronic diseases and conditions like diabetes, heart disease, Alzheimer’s and cancer, potentially reducing the need for drug therapy. Increasing our investments in vaccine research and development now – especially the basic research that leads to new discoveries – is a wise, strategic investment choice and the key to reaping these health and economic benefits.

We would like to acknowledge and thank our sponsors, Senators Tom Harkin and Lamar Alexander, for making this briefing possible, as well as members and staff of the HELP Committee. We would also like to acknowledge and thank Harvey Fineberg, former president of the Institute of Medicine, for his early support, as well as our speakers and the many volunteers who believe in vaccines and work tirelessly behind the scenes to promote their benefits. We are delighted to chair this briefing and we are most grateful to Stanley Plotkin and Adel Mahmoud for organizing an excellent programme of speakers.

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Fast forward to the next generation of vaccines

THE DAWN OF A NEW AGE

In the last 5 to 10 years we have witnessed a remarkable series of advances in science and technology, and nowhere more prominently than in the field of molecular biology and in the knowledge gained for the development of new or improved vaccines. Some of these advances have been groundbreaking; others have been incremental. Combined with a better understanding of the host immune responses that protect against infection, transmission or spread of infectious agents, these advances are paving the way for the development of a new generation of vaccines to prevent diseases and infections for which we have never had vaccines, such as HIV/AIDS, malaria, leishmaniasis, dengue fever, Chikungunya, respiratory syncytial virus (RSV), Ebola, or only partially effective vaccines, like TB and influenza.

ADVANCES IN STRUCTURAL BIOLOGY

Many experts consider advances in structural biology to be among the most significant of these advances because of the potential to revolutionize the way that vaccines are designed. Structural biology provides atomic-level details about the three-dimensional organization and chemical structure of proteins of viruses and other pathogens. A better understanding of how pathogens work – combined with advances of “in silico” protein design – provide a means by which to manipulate, optimize or rationally design immunogens for vaccines. A combination of advances in X-ray crystallography, electron microscopy, high-tech engineering and computer technology has enabled scientists to unlock the secrets of the intricate structure of proteins in target viruses or other pathogens that can be used to guide vaccine design.

STRUCTURE-GUIDED VACCINE DESIGN

This approach is already being used to develop vaccines for infectious diseases. One example is the development of a vaccine for RSV, a highly prevalent respiratory virus that infects lungs and airways. Most healthy people recover from RSV infection in about 1 to 2 weeks. However, infection can often be severe in infants, young children, and older adults. RSV is the most common cause of bronchiolitis (inflammation of the small airways in the lung) and pneumonia in children under 1 year of age in the United States, and the leading cause of hospitalization for all children under 5 years of age. Previous attempts to develop an effective RSV vaccine have failed owing to issues with the stability, purity, reproducibility and potency of the vaccine.

Using novel approaches to discover new classes of monoclonal antibodies (mAbs) and state-of-the-art structural biology techniques, scientists set out to engineer a novel RSV-like viral antigen that provides greater protection than previous RSV experimental vaccines. The goal was to see if focusing on the newly discovered neutralization (NT)-sensitive antigenic site of the virus – dubbed antigenic site zero (Ø) – they could overcome the problems with earlier vaccines. Antigenic site Ø is only present on the prefusion conformation of the RSV fusion (F) glycoprotein. The site was discovered by solving the structure of the F protein in its native trimeric state in complex with the new potent monoclonal antibodies. With the atomic level structure and defining the binding site of these antibodies, structure-based design techniques yielded stabilized versions of the F protein that maintained antigenic site Ø when exposed to extremes of pH, temperature and other conditions. Multiple crystal structures were solved to ensure that modifications in F protein maintained the desired conformation. Based on the immunogenicity of site Ø-stabilized variants of RSV F in mice and rhesus macaques, the stability, and expression levels, a leading RSV candidate vaccine was selected for clinical grade manufacturing. Preparations are underway for a Phase 1 clinical

ADVANCES IN SCIENCE AND TECHNOLOGY

1. Gains in structural biology and structure-guided vaccine design
2. Advances in systems biology to identify and characterize protective immune responses
3. Progress in DNA vaccines, development of new platforms (mRNA), and novel delivery mechanisms (electroporation)
4. Improved vectors and adjuvants
5. Breakthroughs in reverse genetics
6. Discovery of potent, broadly neutralizing antibodies and debut of rational antibody design
7. Development of nanoparticles
8. Emergence of gene therapy approaches
9. Advances in manufacturing, such as cell-based production of influenza vaccines
The excitement generated by this triumph of structure-based vaccine design – the creation of a stabilized prefusion glycoprotein vaccine to prevent RSV infection – offers the hope that this technique can be employed to design vaccines for other diseases and infections that have resisted vaccine development efforts by conventional methods.

APPLICATIONS FOR HIV VACCINE DESIGN

Many viruses, bacteria and other pathogens, such as HIV, have evolved multiple mechanisms to evade neutralizing antibody responses. In the case of HIV, the enveloped protein evades host recognition by virtue of its sequence diversity, its limited exposure to the immune system because of carbohydrate “masking” by sugars, and its conformational flexibility. HIV has been resistant to classification by traditional serotyping, suggesting that standard approaches to vaccine development are unlikely to succeed. Recent progress in the definition of highly potent, broadly neutralizing antibodies that could prevent or contain infection.

THE PROMISE OF DNA VACCINES

The delivery of DNA or RNA for the in vivo production of antigen was first reported more than two decades ago for both prevention of disease and as immune therapy for patients already infected. The attraction of these platforms originated from a paradigm shift engendered by “nucleic acid-based vaccine” (NAV) technology. NAV technology enables indefinite repeat boosting because they are not subject to neutralization by the host immune response, thus facilitating boosting even in infected individuals.

Great strides have been made in the development of synthetic DNA vaccines over the last fifteen years. Advances in technology have made it possible to make synthetic DNA relatively easily and quickly: strands of synthetic DNA can be made in a couple of hours: a gene can be synthesized in a couple of days: while the genomes of poliovirus or influenza A virus would take only a matter of weeks. Making synthetic versions of small bacterial genomes is no longer an impossibility and are being pursued. After the first human clinical studies of DNA vaccines established their initial safety and immunogenicity, there has been a resurgence of interest in the DNA vaccine field due to the development of second-generation synthetic “enhanced” DNA vaccines and delivery approaches, which have radically improved this technology. These new synthetic DNA vaccines exhibit improved immune potency in humans; and, for the first time, have demonstrated clinical efficacy, specifically reversing cervical disease, which if left untreated can lead to cancer in women.

As the name suggests, DNA vaccines consist of DNA sequences that code for a particular antigen, which is directly injected into the individual’s muscle. The DNA inserts itself into cells, which then produce the antigen from the infectious agent. Since this antigen is foreign, it generates an immune response. This type of vaccine has the benefit of being relatively easy to produce, since DNA is very stable and easy to manufacture. Despite consisting of genetic material, large numbers of clinical trials have shown this innovative approach to be safe. For the moment there is no licensed DNA vaccine for humans.

ELECTROPORATION

This new delivery technique, dubbed electroporation, is fast becoming the preferred method for delivering DNA vaccines and enabling immune responses to the target pathogen. Most drugs and all genes – including DNA-based vaccines – used for medicinal benefit act on existing “machinery” inside a cell to perform their intended function.

Advances in electroporation technology have shown a preeminent ability to safely and effectively deliver DNA-based vaccines. Numerous human studies have demonstrated best-in-class immune responses from DNA vaccines delivered using this technique, which uses controlled, millisecond electrical pulses to allow dramatic cellular uptake of a synthetic DNA vaccine, which was previously injected into an individual’s muscle or skin. Studies have shown that electroporation is ideal for inducing the production of antibodies and killer T-cells, which are considered vital for treating many infections and cancers. The combination of DNA vaccines delivered using electroporation devices has to date shown a favorable safety profile, without serious adverse events and only mild local injection-related side effects, such as redness and swelling. Administering electroporation is well tolerated and can be used without an anesthetic; and because it does not induce unwanted immune responses, it can be repeatedly administered for booster vaccinations.

A U.S. biotech company that pioneered the use of DNA vaccines is leading the development of new electroporation devices to produce the desired methods and conditions to deliver synthetic DNA-based vaccines safely, reliably and with a tolerability similar to existing needle injections. Other companies are even developing therapeutic vaccines for cancer.

DEVELOPMENT OF NEW PLATFORMS

Messenger RNA (mRNA) is a large family of single-stranded RNA molecules that are complementary to one of the DNA strands of a gene and convey
genetic information from DNA to the ribosome inside the cell, where they specify the amino acid sequence of the protein products when genes are expressed. Just as good wine improves with age, cutting-edge technologies can take time to mature, and direct injection of RNA for immunization has recently been shown to be significantly improved.

Improved methods for mRNA synthesis and stabilization, and the development of self-amplifying RNA, have yielded promising results in animals over the past year. Through optimization of the GC (guanine-cytosine) content of the synthetic mRNA – high GC-content is more stable than low GC-content – and by complexing the mRNA with a small nuclear protein, called protamine, to protect the mRNA from destruction, the in vivo performance of this platform was significantly improved. Furthermore, the vaccines induced immune responses in larger animal models that resulted in disease attenuation upon challenge with virus. The results imply that such an mRNA approach could allow for more rapid generation of new influenza vaccines, in particular.

The RNA immunization field has been extended by self-amplifying RNA technology. This strategy uses what are called “alphavirus replicons,” which are parts of the virus replication machinery that can incorporate foreign nucleic acids but which are deprived of the ability to synthesize the virus. By delivering the alphavirus genes encoding the RNA replication machinery along with the recombinant viral target antigens, very low doses of RNA can result in the generation of strong antigen-specific immunity.

RNA approaches have conceptual advantages as well as disadvantages in relation to other vaccine technologies. They are simple, can be delivered directly into the cytoplasm, and do not require nuclear localization to generate expression. Self-amplifying mRNA vectors expand replication by providing the replication machinery for the RNA to increase copy number; they also provide a unique target for host immunity to zero in on in the replication-machinery proteins themselves. Stabilized mRNA approaches have other advantages, such as longer half-life, which generates longer expression time, and formulations that are simpler than those of viral vector systems. Conversely, RNA approaches strongly stimulate the host’s innate defense system. They do this, in part, through activation of the TLR3 and TLR 7/8 pathways.

The place of RNA immunization in relation to DNA immunization is still being worked out, but both strategies allow the host to generate immune responses without requiring in vitro synthesis of the proteins that are required to stimulate immunity in vivo.

For every disease and infection for which we have a vaccine there are at least two other major diseases and infections for which we do not have vaccines or only partially effective vaccines.
NOVEL DELIVERY MECHANISMS
When most people think of vaccination, whether in this country or overseas, they probably think of a doctor or nurse administering a shot with a syringe or needle. Most vaccines against childhood diseases are still delivered this way; and for adults and adolescents, vaccines for the seasonal flu are usually delivered by this method. However, future immunization delivery methods, such as electroporation for the delivery of DNA vaccines, may be quite different from what we use today. Inhaled vaccines, for example, are already used in some cases: influenza vaccines have been made in the form of a nasal spray. One of these vaccines is available every year for seasonal flu. The oral polio vaccine, the preferred polio vaccine for use in developing countries, is administered orally. Novel delivery mechanisms in development include a vaccine patch application, where a patch containing a matrix of extremely tiny needles delivers a vaccine without the use of a syringe. This method of delivery could be particularly useful in remote areas or resource-limited settings, as its application would not require delivery by a trained nurse or other medical professional, which is generally needed for vaccines delivered as a shot by syringe.

NANOPARTICLES AS DELIVERY VEHICLES
Candidate vaccines often fail because of their inability to evoke appropriate immune responses. This is especially true where cellular immunity is required for protective immunity, a problem compounded by the move toward devising subunit vaccines. Over the past decade, ultra small, “nanoscale size” materials, such as virus-like particles (VLPs), liposomes, immune stimulating complexes (known as ISCOMs), polymeric, and non-degradable nanoparticles have been studied as potential delivery vehicles for vaccine antigens which can both stabilize vaccine antigens and act as adjuvants. Importantly, some of these nanoparticles are able to enter antigen-presenting cells (APCs) by different pathways, thereby modulating the immune response to the antigen. This may be critical for the induction of protective cell-mediated, Th1-type immune responses to intracellular pathogens. Their properties also make them suitable for the delivery of antigens at mucosal surfaces and for intradermal administration. Scientists are assessing the utilities of different nanoparticle systems for the delivery of subunit vaccines and evaluating the potential of these delivery systems for the development of new vaccines against a wide range of pathogens. The human papillomavirus (HPV) vaccine, the first virus-like particle (VLP) vaccine licensed for use in the United States, represents an important step forward in the use of nanoparticles.

ADVANCES IN USE OF VECTORS
The experimental use of vectors to deliver vaccines against infectious diseases has become a science in the last 15 years. Vaccine vectors are microbes, usually viruses or bacteria, which have been adapted to accommodate an extra gene from another microbe, the target infectious agent, which encodes a protein crucial to inducing a powerful immune response against that microbe. The majority of vectors are of viral origin, either based on viruses that are not harmful to humans or based on viruses that have been attenuated so they do no harm. Vectors presently in consideration are based on various adenoviruses, measles, Sendai and poliovirus genomes. Bacterial vectors are also under study, for example, based on Salmonella, Shigella and Listeria strains.

For some infectious agents against which a vaccine is needed, it is too dangerous to use the whole microbe in a vaccine. Hepatitis B and human papillomaviruses (HPV) are two such viruses. However, it is known which particular protein could provide immunity. In that case vaccine developers may use another infectious agent as carriers for the protein. These carriers, called vectors, are themselves viruses or bacteria that are either naturally harmless to humans or have been engineered by molecular biology to be unable to fully reproduce. Into those vectors scientists insert the genes for a protective protein. When the vector is injected it reproduces either in part or fully and it also produces the protein that could immunize the host against the target pathogen. An example of this is the use of a poxvirus (related to smallpox but harmless), such as canarypox virus, which can enter human cells but cannot survive and multiply in human cells, to carry a protein from HIV. Another example is the use of respiratory viruses from apes that do not harm humans but can carry proteins from human respiratory viruses and thus induce immunity in humans after injection.

Vectors are attractive to vaccine developers because microbes are usually very good at inducing strong immunity. Moreover, they recruit both adaptive and innate immunity, which results in long-term “memory.” Eliciting a durable response – or long-term memory – is one of the most important goals when developing any new or improved vaccine. Vectors can be selected in favor of cellular
or antibody responses. They can be easily produced, analyzed and have favorable safety profiles. They can be adapted to include molecular adjuvants. However, their utility can be limited by preexisting immunity to the vector itself or when a vaccine boost is necessary.

Adenoviruses are among the many viruses being investigated as vectors. Recombinant adenoviral vectors have demonstrated immunogenicity and protective immunity in a variety of animal models. These adenoviruses have been genetically modified so they can deliver and express specific recombinant gene products, while ensuring that they are unable to grow and replicate on their own. Viral vectors attenuated in this way are called “replication defective” or “replication incompetent.” Adenoviral vectors carrying genes that encode HIV and other viral proteins have been developed and shown to be safe in Phase 1 and 2 clinical trials. Numerous attenuated poxvirus vectors have also been developed and have been widely studied.

Among the live bacterial vectors used for antigen delivery, there are mucosal pathogens that have been attenuated, including strains of Listeria, Salmonella, Vibrio cholera, Shigella, Mycobacterium bovis, Yersinia enterocolitica, and Bacillus anthracis. In addition, there are “commensal” strains of bacteria that are being used as vectors. These bacteria are universally present in humans and considered to be harmless – or, in some case, even beneficial. Streptococcus gordonii, lactobacilli, and staphylococci have been used as vectors for the induction of humoral and cellular responses.

Although substantial work has progressed in animal models and demonstrated vaccine efficacy using viral and bacterial vectors, the ultimate value of gene-based vaccination has yet to be shown in large-scale human studies. Several trials using the poxvirus technology have advanced into clinical evaluation. While it is likely that the licensure of a gene-based vaccine for humans remains several years in the future, the recent approval of two DNA-based vaccines for veterinary use – against West Nile virus in horses, and against a virus called infectious hematopoietic necrosis virus (IHNV), which causes disease in salmon and trout – demonstrate that gene-based vaccines work and suggest that viral vectoring of genes for use in humans is not far away.

**REVERSE GENETICS: BUILDING VACCINES PIECE BY PIECE**

A new technology that is looking increasingly promising for the development of influenza vaccines is so-called plasmid-based reverse genetics, a technique that could speed up the process by which a vaccine is created. With reverse genetics, scientists can custom-make a flu vaccine by assembling genes that code for the desired features. For example, two genes representing the influenza HA and NA antigens are selected from the target influenza virus, while the remaining six genes come from an influenza virus that has been time-tested for its ability to grow inside an egg. The influenza hemagglutinin (HA) and neuraminidase (NA) are glycoproteins found on the surface of influenza viruses that enable the virus to get into and out of the host cell and are indispensable for virus replication. Although influenza viruses use RNA as their genetic material, researchers use DNA because it is easier to work with. The DNA that codes for the desired viral genes is introduced into animal cells, which make new copies of the influenza virus. Researchers recover the resulting virus – sometimes called the “vaccine virus” – that will be used to manufacture the vaccine. Although the vaccine still needs to be grown inside eggs for large-scale production, animal cells could also be used as that technology advances. One key benefit of the new reverse genetics technology is that if portions of a targeted virus, such as the H5 and H7 antigens on avian influenza (bird flu) viruses, are too toxic to grow inside eggs, which is the case with highly pathogenic H5N1 avian influenza viruses, which are lethal to chickens, the segments of the genes coding for these antigens that make them so dangerous can be clipped and removed. Because of this unique characteristic, researchers are using reverse genetics to attempt to develop a vaccine for the H5N1 virus and other avian influenza viruses, in the hope that reverse genetics technology may prove a quick and effective way for developing a suitable vaccine in the event of a human influenza pandemic.

**UNIVERSAL FLU VACCINE**

The scientific knowledge gained from reverse genetics experiments conducted with influenza viruses – combined with knowledge gained from ongoing laboratory research targeting highly conserved epitopes of influenza viruses, such as the stem of the hemagglutinin – could one day contribute to the development of a universal flu vaccine. The search for a universal flu vaccine that would be effective against all seasonal flu strains, thereby doing away with the need for an annual flu shot, is considered one of the holy grails for vaccine developers (the search for an HIV vaccine, an improved TB vaccine, and a malaria vaccine being the other three). Such a universal flu vaccine could theoretically also be effective against pandemic influenza strains.

Reverse genetics has led to important advances in our understanding of viral gene function and interaction with host cells. The technology has also helped scientists identify and further characterize the important determinants of viral transmission and virulence. Since many severe viral human and animal pathogens are RNA viruses, including those responsible for Ebola, measles, mumps and influenza, reverse genetics is also an extremely powerful technique with important potential applications for the prevention and control of a range of human and animal viral diseases.

In particular, reverse genetics offers new approaches for the development of novel live-attenuated virus candidates, based on the generation of genetically engineered viruses from complementary DNA (cDNA) clones synthesized from a messenger RNA (mRNA) template. Stabilized recombinant viruses produced in this way would be better virus candidates for developing vaccines. The technology also provides a reliable tool to identify and remove transmission and virulence determinants in RNA viruses.

This technology could be applied to the development of new vaccines for several RNA viral diseases that have some of the same characteristics as influenza viruses: many different subtypes, high variability and high mutation rates, resulting in constant antigenic changes. Investigators are applying the techniques learned from influenza experiments to see if a stabilized dengue virus could be engineered as a vaccine virus to pursue.
EMERGENCE OF GENE THERAPY APPROACHES

Many of tomorrow's vaccines will contain a gene therapy component, blurring the lines between what constitutes a "vaccine" in the conventional sense of the word and "gene therapy," which most people take to mean something completely different.

A vaccine is a biological preparation designed to provoke an immune response in a vaccinated individual that will provide immunity to a particular disease or infection. A vaccine typically contains an agent that resembles a disease-causing organism and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins. Vaccines stimulate the body's own immune system to recognize an infectious agent as foreign, neutralize it, and keep a memory of it, so the immune system can more easily recognize and destroy any future organism that it later encounters.

Gene therapy is defined as the use of DNA as a "drug" to treat disease by delivering therapeutic DNA into a patient's cells. The most common form of gene therapy involves using DNA that encodes a functional, therapeutic gene to replace a mutated or defective gene. Other forms involve correcting a mutation, or using DNA that encodes a therapeutic protein drug (rather than a natural human gene) to provide treatment. In gene therapy, DNA that encodes a therapeutic protein is packaged within a vector – usually a genetically engineered virus – which is used to deliver the therapeutic DNA inside cells within the body. Once inside, the DNA becomes expressed by the cell machinery, resulting in the production of therapeutic protein, which in turn treats the patient's disease.

The distinction between what constitutes a vaccine and a gene therapy began to blur following the introduction of DNA vaccines, which use recombinant viral vectors engineered to carry a gene from a pathogen to trigger an immune response. The consensus to date has been that any approach that relies upon the body's own immune system to mount an immune response against an infectious agent is a vaccine, whereas any intervention that bypasses the natural immune system is gene therapy.

That neat distinction looks set to change.

One novel vaccine approach takes this process one step further. It involves the transfer of human genes encoding highly potent, broadly neutralizing antibodies known to protect against transmission of an infectious agent, such as HIV, malaria, or influenza, which are produced too late during the course of natural infection to block transmission or to change the course of infection once a foothold has been secured.

ANTIBODY GENE TRANSFER METHOD

The daunting challenges that face us in developing a safe and effective vaccine to prevent HIV infection are well known. Although conventional approaches to generating humoral immunity against HIV have made great strides in the last few years with the precise identification of neutralizing epitopes common to diverse strains of virus, there is still a yawning chasm between these discoveries and turning envelope antigens into protective immunogens. Hence there is the need to consider bold and imaginative ways to leapfrog this barrier. The potent and broadly neutralizing monoclonal antibodies that have recently been isolated...
show promise not only as tools in immunogen design but also as prophylactic agents themselves as shown by passive transfer studies.

One of the most exciting new developments in the HIV field is the application of gene transfer technologies to deliver these antibodies. Advances in the use of gene transfer for the correction of genetic deficiencies, particularly the successful expression of factor IX in a small group of patients with hemophilia B, have bolstered the intriguing possibility of using adeno-associated virus (AAV) vectors as a vehicle for antibody gene delivery in humans. Pioneers of this novel approach to preventing HIV infection have shown that protective monoclonal antibodies, or antibody-like molecules, can be delivered and expressed via gene transfer and have the potential to offer long-term in vivo protection. What has come to be known as vectored immunoprophylaxis (or VIP) circumvents the uncertain immune response inherent in some vaccination regimens and ensures production of the desired protective antibody response.

Antibody gene transfer is a novel vaccine approach that bypasses the natural immune system response that was the target of all previous attempts to develop an HIV vaccine. Because this approach skips many of the steps in the usual path followed by vaccine developers, it has been described as using a leapfrog strategy.

By transferring the human genes that encode for potent, broadly neutralizing antibodies that block HIV infection, and harnessing adeno-associated viruses (AAV) to deliver those genes, investigators have shown they can protect rhesus macaques from SIV infection, and humanized mice (mice with human immune systems) from HIV infection.

The excitement generated by the gene transfer method has revived interest in passive immunization, such as treatment with monoclonal antibodies and other antibody-based therapy options to prevent and treat infectious diseases. It has also sparked renewed interest in the use of AAV as vectors for delivering genes conventional methods. Two teams in the United States working independently of each other have been investigating the use of gene transfer technology for the prevention of HIV and other infectious diseases. After the completion of animal studies which demonstrated the safety and effectiveness of this novel vaccine approach, investigators are now moving forward into human clinical trials.

Many scientific challenges lie ahead that remain to be worked out. These include challenges ensuring that high levels of antibody expression will translate to human patients, and proving that life-long expression of these engineered antibodies are safe. There is also the challenge of convincing funders and policymakers that the antibody gene transfer approach is a valid strategy for vaccine researchers to pursue and one that should not be held back by definitions of what does or doesn’t constitute a vaccine. As we enter a bold new era in vaccine research and development, we should not be blinkered by overzealous adherence to definitions as to what is a vaccine and what is an antibody-mediated microbicid so long as protection is achieved.

The Ebola emergency in West Africa shows that we don’t have vaccines for many diseases that could spread to America. There are at least a dozen Ebola vaccine candidates, but until recently none has entered human clinical trials for lack of financial backing.

to prevent other diseases. AAV are small, ubiquitous, harmless viruses that are structurally simple, versatile and easy to work with. Gene transfer vectors based on recombinant AAV are now being consid-

If these challenges can be overcome, successful demonstration of gene transfer-based immunoprophylaxis promises to alter the landscape of vaccine development and provides a shortcut for tackling other challenging vaccine targets such as hepatitis C, pandemic influenza virus, and malaria. The ever-expanding universe of antibodies that target not only infectious diseases but also aberrant forms of endogenous proteins may lead to the development of entirely new prophylactic and therapeutic interventions that could have a substantial effect on protecting humans from disease.

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The history of vaccines: a short introduction to the different types of vaccines

FIRST GENERATION VACCINES

The first human vaccines were based on using weaker or attenuated viruses to generate protective immunity. In 1796, an English physician, Edward Jenner, took note of the folk observation that milkmaids had smooth complexions: they did not get smallpox. In the nursery rhyme that begins “Where are you going, my pretty maid? I’m going a-milking, sir, she said,” the girl claimed that “My face is my fortune” because it was free of pockmarks. Taking these observations one step further, Jenner conceived the notion of using the relatively harmless cowpox as a “vaccine” (from the Latin vacca, “cow”) to generate cross-immunity to smallpox. Cowpox is a poxvirus similar enough to smallpox to protect against it but does not typically cause serious illness. For many years, Jenner had heard the tales that dairymaids were protected from smallpox naturally after having suffered from cowpox. Pondering this, Jenner concluded that cowpox not only protected against smallpox but also could be transmitted from one person to another as a deliberate mechanism of protection. In May 1796, Edward Jenner found a young dairymaid, Sarah Nelms, who had fresh cowpox lesions on her hands and arms. On May 14, 1796, using matter from Nelms’ lesions, he inoculated an 8-year-old boy, James Phipps, in Gloucester. Subsequently, the boy developed mild fever and discomfort in his underarms. Nine days after the procedure he felt cold and had lost his appetite, but on the next day he was much better. Two months later, Jenner inoculated the boy again, this time with matter from a fresh smallpox lesion. No disease developed, and Jenner concluded that protection was complete. Despite initial misgivings, news of Jenner’s discovery spread rapidly across Europe and the world. After a series of successful mass vaccination campaigns in Europe and North America starting in the 19th century followed by a global eradication push in the 20th century, the WHO solemnly declared smallpox eradicated in 1979.

In 1885, nine decades after Jenner’s discovery of the first live, attenuated vaccine, Louis Pasteur in Paris was inspired to find a way to stabilize and artificially weaken the rabies and anthrax viruses. The notion of using a weak form of a disease to elicit immunity to the virulent version was well known to Pasteur due to the success of Jenner’s smallpox vaccine. The difference between Jenner’s smallpox vaccine and Pasteur’s anthrax and rabies vaccines was that the weakened form of the latter two disease organisms had been generated artificially in the laboratory, so that a naturally weak form of the disease organism did not need to be found. Pasteur produced the first vaccine for rabies by growing the virus in rabbits, and then weakening it by drying the affected spinal cord tissue. His rabies vaccine was the first live, attenuated virus vaccine created in a lab as a vaccine for humans. This discovery revolutionized work in the prevention of infectious diseases. Pasteur gave these artificially weakened organisms the generic name of “vaccines” in honor of Jenner’s discovery, giving birth to a whole new field of medicine, now known as vaccinology.

LIVE, ATTENUATED VACCINES

Nowadays, attenuated vaccines can be made in several different ways. Some of the most common methods involve passing a virus through a non-human host and produce a version of the virus that can still be recognized by the human immune system, but cannot replicate well in a human host. When the resulting “vaccine virus” is given to a human, it will be unable to replicate enough to cause illness, but will still provoke an immune response that can protect against future infection. Protection from a live, attenuated vaccine typically outlasts the protection provided by a killed or inactivated vaccine.

Live, attenuated vaccines currently targeted for use in a vaccine may be grown through – or “passaged” through – upwards of 200 different embryos or cell cultures. Eventually, the attenuated virus will be unable to replicate well (or at all) in human cells, and it can safely be used in a vaccine.

All of the methods that involve passing a virus through a non-human host produce a version of the virus that can still be recognized by the human immune system, but cannot replicate well in a human host. When the resulting “vaccine virus” is given to a human, it will be unable to replicate enough to cause illness, but will still provoke an immune response that can protect against future infection. Protection from a live, attenuated vaccine typically outlasts the protection provided by a killed or inactivated vaccine.

Live, attenuated vaccines currently recommended as part of the U.S. Childhood Immunization Schedule include measles, mumps, and rubella (via the com-
200 YEARS OF DISCOVERY: VACCINE INTRODUCTIONS FROM SMALLPOX TO THE PRESENT DAY


Painting of Edward Jenner courtesy of the Wellcome Library. Photo of Louis Pasteur courtesy of Institut Pasteur.
bined MMR vaccine), varicella (chickenpox), and influenza (in the nasal spray version of the seasonal flu vaccine).

One concern that must be considered is the potential for some attenuated vaccine viruses to revert to a form capable of causing disease. Mutations that can occur when the vaccine virus replicates in the body may result in a more virulent strain. This rarely happens, as the vaccine virus’s ability to replicate at all is limited. However, it is always taken into consideration when developing a new, live attenuated vaccine. It is worth noting that mutations are somewhat more common with the oral polio vaccine (OPV), a live vaccine that is ingested instead of injected. The vaccine virus can sometimes mutate into a virulent form and result in rare cases of paralytic polio. For this reason, OPV is no longer used in the United States, and has been replaced by the inactivated polio vaccine.

**KILLED OR INACTIVATED VACCINES**

One alternative to attenuated vaccines is a killed or inactivated vaccine. Vaccines of this type are created by inactivating a pathogen, typically using heat or chemicals such as formaldehyde or formalin. This destroys the pathogen's ability to replicate, but keeps it “intact” so that the immune system can still recognize it.

The term “inactivated” is generally preferred rather than “killed” to refer to viral vaccines of this type, as viruses are not generally considered to be living organisms since they cannot replicate on their own.

Because killed or inactivated pathogens can’t replicate at all, they can’t cause even mild disease, as can sometimes happen with live, attenuated vaccines. However, inactivated vaccines tend to provide a shorter length of protection than live vaccines, and are more likely to require boosters to create long-term immunity. Killed or inactivated vaccines on the U.S. Recommended Childhood Immunization Schedule include the inactivated polio vaccine and the seasonal influenza vaccine given as a flu shot.

**TOXOID VACCINES**

Some bacterial diseases are not directly caused by a bacterium itself, but by a toxin produced by the bacterium. Tetanus is one example: its symptoms are not caused by the Clostridium tetani bacterium, but by a neurotoxin it produces, called tetanospasmin. Diphtheria is another example. Diphtheria toxin is an exotoxin secreted by Corynebacterium diphtheriae, the pathogen bacterium that causes diphtheria. Vaccines for this type of pathogen can be made by inactivating the toxin that causes disease symptoms. These kinds of vaccines are called “toxoids.” A toxoid is a chemically modified bacterial toxin from a pathogenic microorganism, like tetanus or diphtheria, which is no longer toxic but is still antigenic and can safely be used as a vaccine. As with organisms or viruses used in killed or inactivated vaccines, inactivation of the toxin can be done via treatment with a chemical such as formalin, or by using heat or other methods. Toxoids can actually be considered killed or inactivated vaccines, but are sometimes given their own category to highlight the fact that they contain an inactivated toxin, and not an inactivated form of bacteria. Toxoid vaccines on the U.S. Recommended Childhood Immunization Schedule include the tetanus and diphtheria vaccines, which are available in a combined form.

**SECOND GENERATION VACCINES**

**SUBUNIT VACCINES**

Subunit vaccines, like conjugate vaccines, contain only pieces of the pathogens they are designed to protect against. Subunit vaccines use only part of a target pathogen to provoke a response from the immune system. This may be done by isolating a specific protein from a pathogen – a virus, bacterium, fungus or other organism – and presenting it as an antigen on its own without other proteins. The acellular pertussis vaccine and influenza vaccine (in shot form) are examples of subunit vaccines.

Subunit vaccines can also be created via genetic engineering. A gene coding for a vaccine protein is inserted into another virus, or into producer cells in culture. When the carrier virus reproduces, or when the producer cell metabolizes, the vaccine protein is also created. The end result of this approach is a recombinant vaccine: the immune system will recognize the expressed protein and provide future protection against the target virus. The Hepatitis B vaccine currently used in the United States is a recombinant vaccine.

Another vaccine made using genetic engineering is the human papillomavirus (HPV) vaccine. Two types of HPV vaccine are available – one provides protection against two strains of HPV, the other against four strains. Both are made in the same way: for each strain, a single viral protein is isolated. When these proteins are expressed, they assemble into virus-like particles (VLPs). Since these VLPs contain no genetic material from HPV they cannot cause illness. However, they can prompt an immune response that provides future protection against HPV.
CONJUGATE VACCINES

Conjugate vaccines are somewhat similar to recombinant vaccines. They are made using a combination of two different components. Conjugate vaccines, however, are made using complex sugars from the coats of bacteria. These coats are chemically linked to a carrier protein, and the combination is used as a vaccine. Conjugate vaccines are used to create a more powerful, combined immune response: typically the piece of bacteria presented would not generate a strong immune response on its own, while the carrier protein would. The piece of bacteria cannot cause illness, but combined with a carrier protein, it can generate immunity against future infection. The vaccines currently in use for children against pneumococcal bacterial infections are made using this technique.

LIVE RECOMBINANT VACCINES

These vaccines use attenuated viruses or bacterial strains as vectors. A virus or bacterium from one disease is used as a Trojan horse – essentially hijacked – as a delivery vehicle to carry an immunogenic protein from another infectious agent. In some cases this approach is used to enhance the immune response. In other cases, this method is used when giving the infectious agent itself as a vaccine would cause disease. For example, with its high mutation rate HIV cannot be attenuated enough to be given safely as a vaccine in humans because of the risk the attenuated virus could revert – or recombine with another retrovirus – to become a virulent strain that could cause AIDS.

The first step in making a live recombinant vaccine calls for choosing the right carrier virus or bacterium – the so called “vector” – that will be used as the vehicle to deliver the genes that code for immunogens of the target pathogen to the right target cells in the body. Starting with the complete virus or bacterium being considered as a vector, researchers first identify a section of its DNA that is not necessary for replication. One or more genes that code for immunogens of the target pathogen are then inserted into this region. Each gene contains instructions that tell the body how to make a certain protein. In this case, researchers select genes that code for a protein specific to the target pathogen: an immunogen that will generate an immune response to that pathogen. For example, a baculovirus – a virus that only infects insects – can be used as a vector and the gene for a particular immunogenic surface protein of an influenza virus may be inserted. When the modified virus is introduced into a person’s body by vaccination, the immunogen is expressed and presented, generating an immune response against it – and thereby against the pathogen it originates from.

In addition to insect viruses, human adenoviruses have been considered as potential vectors for use in recombinant vaccines, particularly against diseases such as AIDS. The vaccinia virus, which is the basis for the smallpox vaccine, was the first used in live recombinant vaccine approaches. Experimental recombinant vaccinia strains have been designed to deliver protection against influenza, rabies, and hepatitis B, among other diseases.

THE DIFFERENT TYPES OF VACCINES AT A GLANCE

Today’s vaccines are made using a variety or combination of different processes. They may contain live viruses that have been attenuated (weakened or altered so as not to cause illness); inactivated or killed organisms or viruses; inactivated toxins (for bacterial diseases where toxins generated by the bacteria, and not the bacteria itself, cause illness); or merely segments of the pathogen (this includes both subunit and conjugate vaccines).

Live, attenuated vaccines on the immunization schedule include those against measles, mumps, and rubella (via the combined MMR vaccine), varicella (chickenpox), and influenza (the nasal spray version of the seasonal flu vaccine). The schedule includes vaccines of every other major type, each of which requires different development techniques.
THE EXPLORING SCIENCE OF ADJUVANTS

Long considered more of an art than a science, the development of “second-generation” immunologic adjuvants, such as MPL®, MF59® and AS03™, combined with a better understanding of how adjuvants work, have transformed the field of adjuvants virtually overnight. Moreover, the recent identification and definition of Toll-like receptors (TLRs) and other members of the “pattern recognition receptors” (PRRs) family, which control signalling pathways inside cells, has opened up a whole new field of scientific exploration.

Vaccine developers have always sought ways to boost the immunogenicity of candidate vaccines and get them into the right cells in order to trigger the desired immune response; they have also looked for ways to dramatically reduce the amount of antigen needed in a vaccine to induce the desired immune response by making the vaccine uptake inside cells more efficient. Second-generation adjuvants – and newer ones in the pipeline – mean this is fast becoming a reality.

The term adjuvant is derived from the Latin adjuvare, which means to help. They are substances added to vaccines in order to boost host immune responses that induce protection. An effective vaccine stimulates both arms of the immune system: innate immunity and adaptive immunity. Innate immunity occurs within hours, as immune cells recognize an intruder. Adaptive immunity develops over several years to boost host immune responses that lead to immune memory, when cells specific to the pathogen are retained for later use. Vaccines made from weakened or killed pathogens contain naturally occurring adjuvants and can elicit potent protective immune responses.

Alum and some other adjuvants have the capability to bind antigens to form multi-molecular aggregates, which will encourage uptake by APCs. Some adjuvants are also capable of directing antigen presentation by major histocompatibility complexes (MHC).

Generally speaking, adjuvants are useful for antigens such as inactivated, subunit, and recombinant proteins, which can lose some of their immunological strength. For decades it was thought that adjuvants were not required for live attenuated vaccines, which carry the necessary immune-stimulating signals themselves. However, some recent research suggests that adjuvants can have a helper effect on live vaccines, as well.

TYPES OF ADJUVANTS

Vaccine adjuvants exert their effects by different mechanisms. Some adjuvants, such as alum and emulsions, function as delivery systems by generating “deposits” that trap antigens at the injection site, providing slow release of antigen in order to provide continuous stimulation of the immune system. These adjuvants enhance the antigen persistence at the injection site and increase the recruitment and activation of antigen-presenting cells (APCs). The classic adjuvant that has been used for decades is salts of aluminum, often just called “alum.” Alum adjuvants are present in many vaccines and produce higher levels of antibodies than unadjuvanted vaccines.

Alum is found in the DTaP (diphtheria-tetanus-pertussis), HPV and hepatitis vaccines. It is now well understood that the immune system uses pathogen-associated molecular patterns (PAMPs) to activate pathogen-recognition receptors (PRRs) such as TLRs, as well as a host of other recently discovered receptors: RLRs, NODs, and NLRs. These receptors bind various pathogen ligands (ranging from bacterial cell wall and cell membrane components to bacterial or viral nucleotides, to fungal lipids) to trigger different types of immune responses; if combined with an antigen, these receptors can initiate and enhance specific arms of the immune responses to that antigen.

Scientists now know how pathogen components stimulate various cytokine pathways and how these direct different arms of the immune response function. Different TLRs, located on the plasma

THE ADJUVA N T EFFECT: 6 EXTRAORDINARY THINGS THAT ADJUVANTS CAN DO

| 1 | Provide a strong priming response in naive populations, effectively reducing the number of doses required to induce protection |
| 2 | Increase the duration of the immune response |
| 3 | Enhance specific arms of the immune response, such as cell-mediated immunity (CMI), a critical target for many of the remaining infectious diseases for which we do not have vaccines |
| 4 | Increase the breadth of the immune response to variable antigens, enabling broader cross-protection |
| 5 | Enhance the immune response in poorly responsive populations, such as the elderly and immunosuppressed populations |
| 6 | Allow for dose sparing of antigens where antigen supply is limited |
VACCINES: ENTERING A BOLD NEW ERA/SEPTEMBER 2014

Toll-like receptors (TLRs) are a class of proteins known to play a key role in the innate immune system. They are single, membrane-spanning receptors, usually expressed in sentinel cells, such as macrophages and dendritic cells, that recognize structurally conserved molecules derived from microbes. Once these microbes have breached physical barriers, such as the skin or intestinal tract mucosa, they are recognized by TLRs, which activate appropriate immune cell responses. Most mammalian species are estimated to have between ten and fifteen types of TLRs. Thirteen TLRs (numbered TLR1 to TLR13) have been identified in humans and mice, and equivalent forms of many of these have been found in other mammals. The first reported human TLR was described in 1994. Three years later, it was shown that a TLR, now known as TLR4, could, when ligated using antibodies, induce the activation of genes necessary for initiating an adaptive immune response, a seminal discovery that caught the attention of vaccine developers and adjuvant researchers everywhere.

This new generation of TLR-based adjuvants act by inducing the innate immunity component of the immune system, and, if the right TLR is targeted, can help get the vaccine into the right cell that will produce the desired immune response. Increased knowledge of the role and function of TLRs and PRR pathways in the last fifteen years, combined with ensuing advances in the use of new and improved adjuvants to reach target receptors or membrane or intracellularly, respond to different pathogen-derived signals to induce proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, or type 1 interferon, leading to a predominantly Th1 (cell-mediated) response needed to protect against some of the most challenging targets. For these targets, inducing Th2 antibody responses alone may not be enough to prevent against infection or prevent infection from gaining a foothold. Certain TLR2 agonists being studied have been reported to activate both Th1 and Th2 responses.

**TLR-BASED ADJUVANTS**

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**HOW TOLL-LIKE RECEPTORS (TLRs) WORK**

This graphic shows how pathogen components stimulate various cytokine pathways and how these direct different arms of the immune response. Different TLRs, located on the plasma membrane or intracellularly, respond to different pathogen-derived signals to induce proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, or type 1 interferon, leading to a predominantly cell-mediated (or Th1) response. Certain TLR2 agonists can activate antibody (or Th2) responses. Various adaptor molecules (also known as TLR agonists) that activate the TLRs have been identified and are of special interest to vaccine developers because of their potential as adjuvants.

molecules to boost the receptivity of those receptors – small molecule TLR “agonists” – have shown that the design and development of adjuvant systems to enhance vaccine efficacy and reduce the amount of antigen needed to induce an immune response has blossomed in the last decade and become a science.

**TLR AGONISTS**

Adjuvants currently in clinical use enhance humoral responses but the newest adjuvants in clinical and preclinical trials are focused on generating multifaceted immune responses. The PRR pathways are an attractive source of novel, small molecule adjuvants for vaccines due to their ability to induce strong cell-mediated immunity. TLR 7 and 8 “agonists” – chemicals that bind to a receptor and activate the receptor to produce a biological response – have demonstrated potential as vaccine adjuvants, since they directly activate APCs and can enhance both humoral and cellular immune responses.

These new and improved adjuvant systems, when added to recombinant protein antigens, can be fundamental to the development of effective preventive vaccines against complex pathogens, such as malaria, HIV, and tuberculosis, and for special target populations, such as subjects with an impaired immune response, due to age or medical conditions.

**LOOKING TO THE FUTURE**

Based on these discoveries and ensuing scientific advances and new understanding of the immunology, it is possible to recognize today how most of the adjuvants function. This knowledge should allow rapid screening of compound libraries for molecules that bind these receptors and that may have adjuvant activity leading to the rational design of new adjuvants aimed at stimulating specific arms of the immune response. The level of knowledge associated with the mechanism of action of those specific molecules and, as a consequence, the pattern of cytokines induced, should also allow an assessment of the impact of an adjuvant on immunogenicity and the safety of a vaccine. A clear understanding of the pathogenesis of immune-mediated disorders and their triggers will be required to ascertain the potential impact of future adjuvants. For several adjuvants, the mechanism of action remains elusive (such as the saponins) or may present multiple modes of action (such as aluminum salts).

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Reaching for the stars: The future of vaccination

THE SKY’S THE LIMIT

We stand on the threshold of a bold new era in vaccine research and development. For the first time in 100 years it is not too fanciful to dream of having vaccines against many of the 39 major diseases and infections for which we have not had vaccines or only partially effective vaccines. We can anticipate vaccines against some of the most intractable diseases, such as HIV/AIDS, tuberculosis and malaria. We can imagine a universal flu vaccine that would be effective against all strains of seasonal flu, dispensing with the need for an annual flu shot. We can envisage vaccines against dengue fever, chikungunya, leishmaniasis and many other tropical diseases. In the not too distant future, we will have an Ebola vaccine which has been advanced into human clinical trials. We can even allow ourselves to dream one day of having vaccines against cancer and chronic diseases like diabetes, heart disease – even Alzheimer’s.

FUTURE VACCINES

Vaccines of the future can be divided into three broad groups, namely those of the near future (less than 10 years); the medium-term future (10 to 19 years); and the long-term future (20 to 50 years). For the near future, there are some targets which are clearly on the horizon, such as a Vi-conjugate vaccine for typhoid. Just slightly behind will be vaccines for shigella and a common protein vaccine for Streptococcus pneumoniae. Optimists place a vaccine for malaria, one of the “big three” along with tuberculosis and HIV/AIDS, in the medium-term basket.

The sporozoite malaria vaccine RTS-S is closest, but a definitive malaria vaccine will probably also require antigens from other stages of its life cycle. Vaccines for tuberculosis and HIV/AIDS will require more time. A tuberculosis vaccine will be either a re-engineered BCG vaccine; or a molecular vaccine with several protein antigens; or one based on prime-boost strategies. For HIV/AIDS, the partial success of the Sanofi-Pasteur prime-boost vaccine has given some hope. We have discovered and characterized many highly potent, broadly neutralizing anti-HIV antibodies, and the success of monoclonal HIV antibodies provides hope that we can rationally design immunogens and antibodies, alike. New targets have been described in the transitional state of the HIV envelope protein assumed after initial CD4 binding. What will delay the introduction of new vaccines is the high cost of clinical trials. Longer term, the vaccine approach will be successful for autoimmune diseases, such as juvenile diabetes. In the near future we can look forward to more intelligently designed adjuvants based on our increased knowledge of Toll-like receptors and the innate immune system.

If the requisite funds can be found to accelerate vaccine research and development for all infectious diseases and infections, we could potentially look forward to the introduction of many new or improved vaccines in the next decade. In addition to typhoid, the first of these will be vaccines for targets already in advanced development, such as chikungunya virus, Clostridium difficile, cytomegalovirus, dengue virus, Ebola virus, hepatitis E virus, Borrelia burgdorferi (the cause of Lyme disease), norovirus, West Nile virus, and even RSV, once considered a challenge. We even have some candidate vaccines for shigella.

Vaccines for more difficult targets like hepatitis C will probably require more time. Other targets in the sights of vaccine developers include Epstein-Barr virus, the cause of infectious mononucleosis and associated with certain forms of cancer, such as Hodgkin’s lymphoma, Escherichia coli, Campylobacter, Candida, Chlamydia, Cryptosporidium, Haemophilus influenzae (non typeable), Helicobacter pylori, herpesvirus 6, herpes simplex virus, leishmaniasis, metapneumonia viruses, Moraxella (for otitis), Staphylococcus, Group A and B Streptococci, and trypanosomiasis.

The search for vaccines beyond classic infections, such as insulin-dependent diabetes, cancers, and degenerative diseases, will accelerate in the decade ahead with continuing advances in immunology and a better understanding of immune responses.

The discovery and development of new vaccines depends upon continued investments in basic research, vaccine discovery and translational research, backed by continuing advances in molecular biology. The latter has already led to gains in structural biology and structure-guided vaccine design, and the debut of rationally designed immunogens and antibodies. In parallel we have phenomenal advances in reverse genetics, improved vectors and adjuvants, the development of nanoparticles, progress in DNA vaccines, the development of new platforms and novel delivery mechanisms, and the emergence of gene therapy approaches. Taken together, these advances herald an exciting new era in vaccine research and pave the way for the development of new vaccines that we would have thought beyond our reach just ten years ago.
Biographies of organizers, speakers and chairs

Dr. Alejandro Balazs, Ragon Institute (Speaker)
Alejandro Balazs, Ph.D., is Assistant Professor of Medicine at Harvard Medical School and Principal Investigator at the Ragon Institute of MGH, MIT and Harvard in Cambridge, MA. He leads a laboratory dedicated to exploring the fundamental mechanisms by which the immune system prevents the establishment of infection by employing immunological engineering as a tool to dissect the underpinnings of protection mediated by the natural immune system. His group is focused on applying this understanding to the development and implementation of novel technologies that engineer immunity as a novel approach towards preventing or treating infection.

Dr. Balazs attended the University of California at Los Angeles, where he received a B.S. in Microbiology and Molecular Genetics with Honors in 1999. He then joined the graduate program at Harvard Medical School where he earned a doctorate in 2006 for his work describing the unique gene expression pattern of hematopoietic stem cells. He went on to conduct postdoctoral research under the direction of David Baltimore at the California Institute of Technology, where he developed vectored immunoprophylaxis as a means of expressing monoclonal antibodies in vivo and demonstrated the first proof-of-principle for such a strategy against both influenza as well as HIV. In 2014 he joined the faculty of Harvard Medical School where he works as a principal investigator at the Ragon Institute of MGH, MIT and Harvard.

Among his honors and recognitions are the University of California Chancellor Scholarship, the Vice Provost Recognition Award, the UCLA Alumni Scholarship, and the Elma Gonzalez Dean’s Prize for Research. He was a MARC scholar from 1997 to 1999, an amfAR fellow from 2010 to 2012 and is a member of the American Society of Gene and Cell Therapy.

Dr. Victor Dzau, Institute of Medicine (Chair)
Victor J. Dzau, M.D., is the 8th President of the Institute of Medicine. He is Chancellor Emeritus for Health Affairs and James B. Duke Professor of Medicine at Duke University, and past President and CEO of Duke University Health System. Previously, Dr. Dzau was the Hersey Professor of Theory and Practice of Medicine and Chairman of Medicine at Harvard Medical School’s Brigham and Women’s Hospital, and formerly the Chairman of Department of Medicine at Stanford University.

Dr. Dzau has made a significant impact on medicine through his seminal research in cardiovascular medicine and genetics; his pioneering in the discipline of vascular medicine, and recently his leadership in healthcare innovation. His important work on the renin angiotensin system (RAS) paved the way for the contemporary understanding of RAS in cardiovascular disease and the development of RAS inhibitors as therapeutics. Dr. Dzau also pioneered gene therapy for vascular disease. His recent work on stem cell “paracrine mechanism” and the use of microRNA in direct reprogramming provide novel insights into stem cell biology and regenerative medicine.

In his role as a leader in health care, Dr. Dzau has led efforts in health care innovation. His vision is for academic health sciences centers to lead the transformation of medicine through innovation, translation and globalization. Leading this vision at Duke, he and colleagues developed the Duke Translational Medicine Institute, the Duke Global Health Institute, the Duke-National University of Singapore Graduate Medical School, and the Duke Institute for Health Innovation. These initiatives create a seamless continuum from discovery and translational sciences to clinical care, and promote transformative innovation in health.

As one of the world’s preeminent academic health leaders, Dr. Dzau advises governments, corporations and universities worldwide. He has served as a member of the Council of the Institute of Medicine (IOM) and the Advisory Committee to the Director of the National Institutes of Health (NIH), and as Chair of the NIH Cardiovascular Disease Advisory Committee and of the Association of Academic Health Centers. Currently he is a member of the Board of Directors of the Singapore Health System, the Governing Board of Duke-National University Singapore Medical School, and Senior Health Policy Advisor to Her Highness Sheikha Moza (the Chair of Qatar Foundation). He is also on the board of Health Governors of the World Economic Forum.

Among his honors and recognitions are the Gustav Nylin Medal from the Swedish Royal College of Medicine; the Max Delbruck Medal from Humboldt University, Charite and Max Planck Institute; the Commemorative Gold Medal from Ludwig Maximillian University of Munich; the Inaugural Hatter Award from the Medical Research Council of South Africa; the Polzer Prize from the European Academy of Sciences and Arts; the Novartis Award for Hypertension Research; the Distinguished Scientist Award from the American Heart Association (AHA), and the 2010 AHA Research Achievement Award for his contributions to cardiovascular biology and medicine. He has received 6 honorary doctorates.

Dr. Barney Graham, Vaccine Research Center, National Institutes of Health (Speaker)
Barney S. Graham, M.D, Ph.D, is Senior Investigator at the Vaccine Research Center, NIAID, NIH, Bethesda. Dr. Graham is an immunologist, virologist, and clinical trials physician whose primary interests are viral pathogenesis, immunity, and vaccine development. His work is focused on HIV, respiratory syncytial virus (RSV), and emerging viral diseases. After graduating from Rice University, he obtained his M.D. from the University of Kansas School of Medicine in 1979.
He then completed residency and two chief residencies in Internal Medicine, a fellowship in Infectious Diseases, and a Ph.D. in Microbiology & Immunology at Vanderbilt University School of Medicine, where he rose to the rank of Professor of Medicine with a joint appointment in the Department of Microbiology & Immunology. In 2000 he became one of the founding investigators for the NIAID Vaccine Research Center at NIH, where he is now the Deputy Director and Chief of the Viral Pathogenesis Laboratory. He is a member of the American Society for Clinical Investigation and the American Association of Physicians, and a Fellow of the Infectious Disease Society of America and the American Academy of Microbiology. He has authored more than 250 scientific publications and has been on editorial boards for the Journal of Biological Chemistry, Journal of Virology, Journal of Infectious Diseases, and Journal of AIDS. His laboratory investigates basic mechanisms by which T cells affect viral clearance and immunopathology, and how T-cell function can be modulated by vaccination. RSV vaccine development, exploring the structure and function of the RSV F glycoprotein, and defining mechanisms of antibody-mediated RSV neutralization are projects of particular interest.

**Professor Adel Mahmoud, Princeton University (Organizer)**

Adel A. F. Mahmoud, M.D., Ph.D., is Professor in Molecular Biology and Public Policy at The Woodrow Wilson School of Public and International Affairs and The Department of Molecular Biology at Princeton University. He previously served as President of Merck Vaccines, and as a member of the Management Committee of Merck & Company, Inc. His prior academic positions at Case Western Reserve University and University Hospitals of Cleveland spanned 25 years, concluding as Chairman of Medicine and Physician-in-Chief from 1987 to 1998. He is a member of the Board of the Foundation for Vaccine Research.

Dr. Mahmoud’s academic pursuits focused on investigations of the determinants of infection and disease in human schistosomiasis and other infectious agents. Using immunological and molecular techniques, he defined the role of eosinophils in health and disease. In laboratory and field studies in several endemic areas, he developed the scientific bases for strategies to control helmithic infections, which have been adopted globally. At Merck, Dr. Mahmoud led the effort to develop four new vaccines, which were launched in 2005-2006, including: combination of Measles, Mumps, Rubella and Varicella; Rotavirus; Shingles and Human Papillomavirus. Dr. Mahmoud’s leadership in setting strategies for global health shaped the agenda of the Forum on Microbial Threats of the Institute of Medicine, which in recent years tackled topical issues, such as biological threats and pandemic influenza, and other threats. He is an active contributor to scientific literature and has authored and edited several textbooks and reports.

Dr. Mahmoud received his M.D. degree from the University of Cairo in 1963, and his Ph.D. from the University of London, School of Hygiene and Tropical Medicine in 1971. He was elected to membership of the American Society for Clinical Investigation in 1978, the Association of American Physicians in 1980, and the Institute of Medicine of the National Academy of Sciences in 1987. He received the Bailey K. Ashford Award of the American Society of Tropical Medicine and Hygiene in 1983, and the Squibb Award of the Infectious Diseases Society of America in 1984. Dr. Mahmoud is a fellow of the American College of Physicians. He served on the National Advisory Allergy and Infectious Diseases Council and is a past president of the Central Society for Clinical Research, and the International Society for Infectious Diseases. Dr. Mahmoud chairs and serves on the Board of several public and scientific organizations in addition to the Foundation for vaccine Research.

**Dr. Gary Nabel, Sanofi (Speaker)**

Gary J. Nabel, M.D., Ph.D. is Chief Scientific Officer, Senior VP and Deputy to the President, Sanofi Global R&D. Dr. Nabel joined Sanofi in November 2012 from the National Institutes of Health, where he served as Director of the Vaccine Research Center (VRC) of the National Institute of Allergy and Infectious Diseases since 1999. During his tenure at the NIH, Dr. Nabel provided overall direction and scientific leadership of the basic, clinical, and translational research activities of the VRC, and guided development of novel vaccine strategies against HIV and other emerging and re-emerging infectious diseases, including Ebola/Marburg hemorrhagic fevers, influenza, chikungunya and other viruses.

Dr. Nabel graduated magna cum laude from Harvard College in 1975 and continued his graduate studies at Harvard, completing his Ph.D. in 1980 and his M.D. two years later. He then served as a post-doctoral fellow in the laboratory of David Baltimore at MIT’s Whitehead Institute. Before his appointment at the VRC, Dr. Nabel served as the Henry Sewall Professor of Internal Medicine, professor of Biochemistry, and Howard Hughes Medical Institute investigator at the University of Michigan in Ann Arbor. In addition to his faculty positions, Dr. Nabel also served as the Director of the Center for Gene Therapy and co-director of the Center for Molecular Medicine at the University of Michigan.

In recognition of his expertise at the forefront of virology, immunology, gene therapy, and molecular biology, Dr. Nabel was elected to the Institute of Medicine of the National Academy of Sciences in 1998. Among his many other honors, Dr. Nabel received the Amgen Scientific Achievement Award from the American Society for Biochemistry and Molecular Biology, the Health and Human Services Secretary’s Award for Distinguished Service, and is a fellow of the American Association of Physicians, and the American Academy of Arts Sciences.

**Professor Stanley Plotkin, University of Pennsylvania (Organizer and chair)**

Stanley A. Plotkin is Emeritus Professor of the University of Pennsylvania, and Adjunct Professor of the Johns Hopkins University. Until 1991, he was Professor of Pediatrics and Microbiology at the University of Pennsylvania, Professor of Virology at the Wistar Institute, and, at the same time, Director of Infectious Diseases and Senior Physician at the Children’s Hospital of Philadelphia (CHOP). He maintained laboratories at both CHOP and Wistar. In 1991, Dr. Plotkin left the University to
join the vaccine manufacturer, Pasteur-Mérieux-Connaught (now called Sanofi Pasteur), where for seven years he was Medical and Scientific Director, based at Marnes-la-Coquette, outside Paris. He is a founding member of the Foundation for Vaccine Research.

Dr. Plotkin attended New York University, where he received a B.A. degree, and then the State University of New York Medical School in Brooklyn, where he received an M.D. degree in 1956. His subsequent career included internship at Cleveland Metropolitan General Hospital, residency in pediatrics at the Children’s Hospital of Philadelphia and the Hospital for Sick Children in London, and three years in the Epidemic Intelligence Service of the Centers for Disease Control of the US Public Health Service.

He has been chairman of the Infectious Diseases Committee and the AIDS Task Force of the American Academy of Pediatrics, liaison member of the Advisory Committee on Immunization Practices and Chairman of the Microbiology and Infectious Diseases Research Committee of the National Institutes of Health. Dr. Plotkin received the Bruce Medal in Preventive Medicine of the American College of Physicians, the Distinguished Physician Award of the Pediatric Infectious Diseases Society, the Clinical Virology Award of the Pan American Society for Clinical Virology, the Richard Day Master Teacher in Pediatrics Award of the Alumni Association of New York Downstate Medical College, and the Marshall Award of the European Society for Pediatric Infectious Diseases. In June 1998, he received the French Legion of Honor Medal; in June 2001, the Distinguished Alumnus Award of the Children’s Hospital of Philadelphia, in September 2006 the gold medal from the same hospital; the Sabin Gold Medal in May 2002, in September 2004 the Fleming (Bristol) Award of the Infectious Diseases Society of America, in May 2007 the medal of the Fondation Mérieux, in 2009 the Finland Award of the National Foundation for Infectious Diseases and the Hillelman Award of the American Society for Microbiology; and in 2013 the Career Achievement Award from the Association for Clinical and Translational Medicine, as well as the Caspar Wistar Medal of the Wistar Institute of Biological Research. He was elected to the Institute of Medicine of the National Academy of Sciences in 2005, to the French Academy of Medicine in 2007, and to the French Academy of Pharmacy in 2013.

Dr. Plotkin holds honorary doctoral degrees from the University of Rouen (France) and the Complutense University of Madrid (Spain). Named lectures in his honor have been established at the Pediatric Academic Societies annual meeting, at the International Advanced Vaccinology Course in Annecy, France, and at the International Society for DNA Vaccines. A professorship in his name was established at the Children’s Hospital of Philadelphia. His bibliography includes over 700 articles and he has edited several books, including the standard textbook on vaccines, now in its 6th edition. He developed the rubella vaccine now in use throughout the world, is co-developer of the pentavalent rotavirus vaccine, and has worked extensively on the development and application of other vaccines, including anthrax, oral polio, rabies, varicella, and cytomegalovirus.

Professor Robert Tjian, Howard Hughes Medical Institute (Chair)

Robert Tjian, Ph.D., has been president of the Howard Hughes Medical Institute since April 2009. Trained as a biochemist, he has made major contributions to the understanding of how genes work during three decades on the faculty of the University of California, Berkeley. He was named an HHMI investigator in 1987.

Dr. Tjian studies the biochemical steps involved in controlling how genes are turned on and off, key steps in the process of decoding the human genome. He discovered proteins called transcription factors that bind to specific sections of DNA and play a critical role in controlling how genetic information is transcribed and translated into the thousands of biomolecules that keep cells, tissues, and organisms alive. Tjian’s laboratory has illuminated the relationship between disruptions in the process of transcription and human diseases such as cancer, diabetes, and Huntington’s. More recently, he has begun studying how transcription factors control the differentiation of embryonic stem cells into muscle, liver, and neurons.

Tjian received a bachelor’s degree in biochemistry from Berkeley in 1971 and a Ph.D. from Harvard University in 1976. After completing a postdoctoral fellowship at the Cold Spring Harbor Laboratory with James Watson, he joined the Berkeley faculty in 1979. At Berkeley, Tjian assumed a variety of leadership roles, including spearheading a major campus initiative to support and implement new paradigms for bioscience teaching and research. He served as the Director of the Berkeley Stem Cell Center, and the Faculty Director of the Li Ka Shing Center for Biomedical and Health Sciences. He is a member of the National Academy of Sciences and has received many awards honoring his scientific contributions, including the Alfred P. Sloan Prize from the General Motors Cancer Research Foundation and the Louisa Gross Horwitz Prize from Columbia University. He was named California Scientist of the Year in 1994.

Tjian remains an active scientist. His small laboratory group at HHMI’s Janelia Farm Research Campus focuses on the development of new approaches to image biochemical activities in single living cells. He also maintains a research laboratory at UC Berkeley, where he is a professor of biochemistry and molecular biology.

Professor David Weiner, University of Pennsylvania (Speaker)

David B. Weiner, Ph.D., is Professor of Pathology and Laboratory Medicine, Co-Program leader of Tumor Virology at the Abramson Cancer Center, and Chair of the Gene Therapy and Vaccines Graduate Program, at the University of Pennsylvania.

Professor Weiner received his B.S. in Biology from Stony Brook University in NY in 1978. In 1985 he received his M.S. in Biology from the University of Cincinnati, and in 1986 he received his Ph.D. in Developmental Biology from the IDP at the University of Cincinnati. He moved to the University of Pennsylvania as an immunology fellow in 1986 and joined the faculty of the University of Pennsylvania in 1989.

His laboratory helped to found the field of DNA vaccines and along with collaborators was the first to move DNA vaccines into human clinical studies.
establishing their initial safety and immunogenicity. His group has been instrumental in the recent resurgence in interest in the DNA vaccine field due to developing second generation synthetic enhanced vaccines and delivery approaches which have radically improved this technology. These new synthetic vaccines exhibit improved immune potency in humans and for the first time demonstrated clinical efficacy, specifically reversing cervical disease, which if untreated can lead to cancer in women. His laboratory has published 350+ peer reviewed scientific research publications, reviews and 8 books/special volumes. Dr. Weiner has been awarded over 60 patents. He is an avid supporter of scientific education and graduate and post-graduate student training. He has served on advisory boards, as well as a consultant to academic organizations or industry. His service includes work with vaccine important organizations such as the NIH, FDA, NIBSC, WHO, EU Commission, Bill & Melinda Gates Foundation, Pfizer, Inovio, BMS, Medimmune, Novartis, Merck, J & J, and VGX among others. Dr. Weiner, among other honors, was elected a fellow of the American Association for the Advancement of Science in 2011, an elected fellow of the International Vaccine Society, and is an NIH Directors Transformative Research Award Winner.
The Foundation for Vaccine Research is an independent advocacy and campaign organization based in Washington, DC, dedicated to advancing vaccine research against infectious diseases. Our mission is to educate, inform and persuade a wide range of audiences, including governments, grant-making institutions, donor organizations, investors, policymakers and other decision makers — as well as the media and civil society — of the benefits of investing in vaccine research and development, and to help mobilize the resources that scientists need to build on advances and pursue promising lines of research.

We accomplish our mission by combining information, education and communication programs with special events and activities, by promoting vaccine research and its benefits to a global audience, by hosting and organizing scientific meetings, by conducting research updates and briefings, by providing a source of unbiased scientific information and strategic advice for policy, by fostering international cooperation, and by making targeted, high-yield investments in time and capital to speed progress, unlock resources, mobilize new assets, and spur scientists on towards reaching their goals.