

on Vaccine Research

ABSTRACTS OF INVITED PRESENTATIONS

1

Vaccines Against Bioterrorism

P. K. Russell

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Vaccines have an important role in protection of the civilian population against the threat of bioterrorism. Recent events have heightened level of concern about bioterrorism and have created an urgent need for increased stockpiles of existing vaccines and accelerated development of new vaccines. This is a challenge to the government, the vaccine industry and the scientific community. National needs include a larger stockpile of smallpox vaccine, an improved smallpox vaccine for high risk populations, a larger national stockpile of anthrax vaccine and an improved second generation anthrax vaccine. Programs are underway to fill those and other needs for an effective civilian biodefense. The optimal utilization of smallpox and anthrax vaccines in civilian defense against biological is a difficult problem and the subject of broad public debate. Both smallpox (vaccinia virus) vaccine and anthrax vaccines can be used to vaccinate high risk populations and for post-exposure management of a bioterrorist event.

Limited vaccine supply determines current policy but, as the stockpiles become larger, future policies will require careful analysis and thorough consideration of the risks, costs and benefits of the policy options.

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2

Tuberculosis: Progress in the Development of a New Vaccine Against the Captain of All the Men of Death

M. A. Horwitz

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Tuberculosis continues to ravage humanity, causing 2 million deaths per year worldwide. A vaccine more potent than the current vaccine, *Mycobacterium bovis* BCG, is sorely needed. We have developed a recombinant BCG vaccine that is more potent than BCG in the highly demanding guinea pig model of pulmonary tuberculosis, a model noteworthy for its resemblance to human tuberculosis. The recombinant vaccine, rBCG30, overexpresses the 30 kDa major secretory protein (a mycolyl transferase) of *Mycobacterium tuberculosis*, the primary causative agent of tuberculosis (Horwitz et al. PNAS (USA) 97:13853, 2000). The rationale for the vaccine derives from the Extracellular Protein Hypothesis for vaccines against intracellular pathogens, which holds that proteins that are secreted or otherwise released from intracellular pathogens such as *M. tuberculosis* are key immunoprotective determinants (Blander & Horwitz. J. Exp.

Med. 169:691, 1989; Horwitz et al. PNAS (USA) 92:1530, 1995). Ten weeks after challenge with a large aerosolized dose of virulent *M. tuberculosis*, rBCG30-immunized animals have significantly fewer *M. tuberculosis* organisms in their organs (0.5 logs fewer in their lungs and 1 log fewer in their spleens) and significantly less lung, liver, and spleen pathology than parental BCG-immunized animals. When monitored for survival after challenge, animals immunized with rBCG30 have significantly longer survival than BCG-immunized animals. The rBCG30 and parental BCG vaccines are comparably avirulent in guinea pigs. The rBCG30 vaccine is currently being readied for Phase I human clinical trials.

4

B Cell Memory and the Persistence of Antibody Responses

I.C. M. MacLennan

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Established T cell-dependent antibody responses can be extremely long lived. This is due in part to long-lived of plasma cells that are mainly found in the red bone marrow, but also to the reactivation of persistent memory B cell clones. The existence of both of these components of long-term responses have been demonstrated by cell transfer experiments between congenic strains of rodents. Examples of these experiments will be described.

T cell-dependent antibody responses characteristically mature slowly and repeat exposure to antigen characteristically is required to reach peak affinity of the antibody and high antibody titres. Much is known of the way affinity maturation in these responses occurs by hypermutation of the genes encoding the antigen-specific receptors of B cells proliferating in germinal centres. Critical to this process is a stage in which B cells are subsequently selected, either to become long-lived plasma cells or memory cells. These processes will be outlined.

Truly T cell independent antibody responses can be induced either by strong cross-linking of B cell receptors alone, as can be achieved with certain bacterial capsular polysaccharides, or antigens that cross link innate immune receptors on B cells with the B cell receptors. The latter typically occurs in responses to bacterial outer cell wall lipopolysaccharides. The characteristics and cellular and molecular basis of these T-independent responses will be compared with those induced by T cell-dependent antigens, using responses to vaccines as examples.

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5 Role of Memory in Vaccine Induced Protection

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To determine the relative importance of vaccine-induced memory cells versus that of vaccine-induced effectors (antibodies, T cells) requires to define under which conditions the reactivation of memory cells upon pathogen exposure is sufficient to confer protection. Evidence is accumulating that reactivation of HbsAg-specific memory does not prevent viral replication in previously vaccinated individuals without residual antibodies at exposure, but that it subsequently induces viral clearance and prevents establishment of chronic hepatitis B infection. The capacity of glycoconjugate vaccines induced memory to confer protection after disappearance of circulating antibodies to capsular polysaccharides (PS) is a more complex issue. Most conjugate-vaccine primed children remain protected despite subprotective serum antibody titers, as highlighted by the high efficacy of a weakly immunogenic HIB-diphtheria toxoid vaccine and the persistence of a high level of HIB vaccine effectiveness in non-boosted U.K. children or use of DT Pa-HIB based combination vaccines with lower HIB immunogenicity. This does not solely reflect herd immunity at the population level and suggests that the capacity of rapidly mounting high antibody responses following bacterial exposure contributes to protection. The assumption that immune memory plays a major role in protection against meningococcal disease led to implementation of group C conjugate vaccines in the U.K.. As bactericidal vaccine antibodies rapidly decline in the youngest age groups, this will indicate the role of memory-mediated protection when the incubation period may be short and the infecting inoculum possibly high. The identification of the determinants which lead - or not - to the successful induction of protective memory responses in immunocompetent and immunocompromised individuals requires further studies.

7 New Designs for HIV Vaccines: Antigens of Promise for CTL and Broadly Neutralizing Antibodies

G. J. Nabel

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Difficulties in generating effective vaccines for several highly prevalent infectious diseases, including AIDS, malaria and tuberculosis, have underscored the need to advance vaccine development for diseases with a high human health impact. The lack of naturally occurring, protective immune responses in HIV infection poses significant challenges to the effort to develop AIDS vaccines. Approaches to facilitate the development of highly effective AIDS vaccines include identification of immunogens that elicit broadly neutralizing antibodies, better understanding the molecular and cellular basis for immune responses to components of the infectious agent, identification of relevant forms of viral proteins for antigen presentation, stimulation of relevant T cell types, and enhancement of antigen-presenting, dendritic cell function. To identify improved immunogens, our laboratory has investigated the effect of specific mutations in HIV-1 envelope on humoral and cellular immune responses after DNA vaccination. In vivo tests in mice demonstrated that specific mutations enhanced humoral immunity without reducing the efficacy of the CTL response. Progress has been made in eliciting neutralizing antibody responses, though induction of broadly neutralizing antibodies has not yet been achieved. In other studies, immunogens designed to enhance cellular immunity were developed and tested. Immune responses to HIV virion-like structures or a polyprotein were examined after DNA immunization with Rev-independent expression vectors. We found that a Gag-Pol fusion protein stimulated both CTL and antibody responses to Gag and Pol, while a Gag-Pol pseudoparticle did not elicit substantial Pol responses. These studies demonstrate the potential for engineering effective protective vaccines against HIV.

6 Memory at the Mucosal Level

M. M. Levine

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The ability to administer vaccines by mucosal (mainly oral or nasal) routes rather than by parenteral injection, offers many advantages, particularly for immunizing populations in developing countries. Mucosal vaccination tends to be well accepted (leading to high compliance); some formulations are extremely practical (e.g., Sabin polio vaccine), thereby simplifying logistics; by appropriate manipulation, mucosal vaccines can stimulate all effector arms of the immune system. In the past it had been taught that mucosal immunization could not elicit sustained immune responses or long-lived protection. However, it is now accepted that certain mucosal vaccines (e.g., oral cholera and oral typhoid) can stimulate enduring protection. For example, in large-scale controlled field trials in Santiago, Chile, 3 doses of Ty21a live oral typhoid vaccine conferred significant protection that endured up to 7 years.

The mechanisms responsible for B cell memory at the mucosal level are being increasingly revealed and are helping to explain the clinical and epidemiologic observations. It is now believed that long-lived memory B cells sustain protective immunity at the mucosal level, even without repeated subsequent exposure to antigen. Differences in the immune response of groups of subjects who are immunologically-naïve, recently immunized or immunized in the more distant past with mucosal vaccines will be discussed.

8 Recent Primate Trials - Clear Forks in the Path Towards an AIDS Vaccine

H. L. Robinson

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Multiprotein DNA Priming and Recombinant Poxvirus Boosting for an AIDS Vaccine

Harriet L. Robinson, Yerkes Regional Primate Research Center and VRC of Emory University

Atlanta, GA 30322

We have showed that DNA priming plus recombinant poxvirus boosters are more effective than DNA alone or DNA plus protein boosters in raising protective immunity against SHIV challenges. To further define parameters for a DNA/poxvirus vaccine, a preclinical trial was undertaken by the Emory Vaccine Research Center, NIAID, and CDC. Rhesus macaques were immunized with 2.5 mg or 0.25 mg doses of a SHIV-89.6 Gag-Pol-Env or Gag-Pol expressing DNA at 0 and 8 weeks and boosted with a SHIV-89.6 Gag-Pol-Env or Gag-Pol recombinant MVA at 24 weeks. One Gag-Pol-Env group received recombinant 89.6 gp120 protein at the 2nd immunization and at the booster. Another Gag-Pol-Env group received GM-CSF DNA at both DNA administrations. Seven months following the booster, the macaques received an intrarectal SHIV-89.6P challenge. Cellular and humoral responses and post-challenge viral RNA levels were measured. The best infection control was achieved in groups receiving the Gag-Pol-Env DNA/MVA vaccine. 19 of 20 animals receiving a high-dose DNA prime have controlled infection at or below detectable levels for >1.5 years. 12 of 12 animals receiving low-dose i.d. DNA prime with or without GM-CSF DNA also have controlled infection at or below the detectable threshold. The Gag-Pol immunogens were not as effective as Gag-Pol-Env; the added gp120 protein also did not provide better infection control. We plan to proceed with a Gag-Pol-Env DNA/MVA vaccine.

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- 9** **The Foot-and-Mouth Disease Epidemic in the United Kingdom, 2001: Why Vaccination was Not Employed**
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surrey, UNITED KINGDOM

On 20 February 2001 an outbreak of foot-and-mouth disease was confirmed in pigs at an abattoir in Essex, England. This was the start of an epidemic which continued until 30 September 2001, reached a total of 2030 outbreaks and resulted in the slaughter of around 6.5 million animals. The cost to the taxpayer has been estimated at around £2.75 billion. Throughout the epidemic the possibility of using vaccination was hotly debated. Among the types of vaccination considered were: (i) ring vaccination around infected foci; (ii) barrier vaccination between infected and free areas; (iii) mass vaccination of cattle and sheep; (iv) vaccination of rare breeds and zoo animals; and (v) vaccination of cattle in "hot spots". The only scheme favoured by the Government was scheme (v) but it was opposed by a significant proportion of the farming community and their national representatives. Those opposed to the scheme argued that the declaration by some retailers that they would label the milk and meat from vaccinated animals would be bound to create a perception among the public that these products posed a risk to health and this would cause a loss of trade. Those opposed to vaccination also argued that it would result in extended embargoes on the export of livestock and animal products and thus be very costly. The Government decided that without the full co-operation of the farming community the vaccination scheme would not succeed and so they decided not to implement it.

- 11** **Vaccination Against Respiratory Diseases of Poultry: Avian Influenza and Newcastle Diseases**
D. E. Swayne
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In the USA each year, 8.2 billion commercial chickens are vaccinated against common respiratory pathogens such as Newcastle disease (ND) virus, a paramyxovirus type 1, and infectious bronchitis virus, a coronavirus. Most commercial poultry are vaccinated through mass immunization programs utilizing live virus vaccines typically given by aerosol spray or drinking water application. However, some inactivated whole virus or recombinant vaccines are used and are administered by injection of individual birds. Vaccination against avian influenza (AI) virus, a type A orthomyxovirus, is uncommon.

Most ND and AI viruses produce low virulent respiratory or reproductive syndromes while a few high virulent strains cause high mortality systemic disease. The latter, velogenic ND and highly pathogenic AI, are exotic to the USA and impact international trade in poultry and poultry products. The ND and AI vaccines protect against all clinical forms.

Various vaccines technologies are effective in immunization against AI and include conventional inactivated AI vaccines, vectored viruses, subunit proteins and DNA vaccines. These vaccines protect from clinical signs and death, and reduce replication of field virus with a homologous hemagglutinin subtype. Currently, inactivated whole AI virus vaccines and a fowl pox vectored vaccine with AI H5 hemagglutinin gene insert are used commercial in various countries of the world. Since 1995, over 1.2 billion doses of inactivated H5N2 vaccine and 500 million doses of recombinant fowlpox-AI H5 vaccine have been used in Mexico in chickens. Hemagglutinin subunit protein, DNA and recombinant ND virus-AI hemagglutinin gene insert vaccines show potential as the next generation of AI vaccines.

- 10** **Novel Strategies to Control Foot-and-Mouth Disease**
M. J. Grubman, J. Chinsangaram, M. P. Moraes
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Greenport, NY

The recent outbreak of foot-and-mouth disease (FMD) in the United Kingdom and its re-emergence in parts of South America has rekindled concern in FMD-free countries to the threat of this highly infectious disease. Currently the method of disease control in FMD-free countries is slaughter of infected and in-contact susceptible animals sometimes in combination with vaccination. The current vaccine is a chemically inactivated virus, but concerns with its use have limited its application in disease-free countries. Furthermore, vaccination requires a number of days to induce protection, a problem during an FMD outbreak when it is essential to rapidly limit the spread of the disease. To address these concerns, we have developed a combination control strategy. We have constructed genetically engineered FMD vaccines containing only the viral capsid and 3C protease coding regions from a number of FMDV types in a replication-defective human adenovirus and have evaluated their potency and efficacy in animals. The recombinant virus is safe, does not spread to uninoculated animals, and swine given a single high dose of Ad5-FMDVA24 are protected from direct inoculation challenge 7, 14, or 42 days postvaccination. To protect animals prior to the induction of the adaptive immune response elicited by vaccination, we have produced an adenovirus containing a porcine cytokine gene. Administration of this virus rapidly protected swine from clinical signs of disease and viremia after direct inoculation exposure to virulent FMDV.

These novel studies suggest that a combination strategy can be successfully used in FMD-free countries to induce immediate and long-term protection.

- 12** **Vaccination of Poultry: the Special Case of Marek's Disease - Herpesvirus-induced Lymphomas**
R. L. Witter
Avian Disease and Oncology Lab, USDA-ARS, East Lansing, MI

Vaccines are the cornerstone of programs to control infectious diseases in chickens. Marek's disease (MD) is caused by a cell-associated alpha herpesvirus and is characterized by lymphomas, neurological disease and immunosuppression. The disease has a rapid onset and can result in up to 100% mortality. Licensed vaccine strains from all 3 MD viral serotypes are in use. There are several unique features of MD vaccines. Vaccines typically consist of cryopreserved suspensions of infected cell cultures and are administered to virtually all commercially-reared chickens. Vaccines are administered by inoculation at hatch or by inoculation into the amniotic sac of 18-day embryos. Immunity is usually well established 5-7 days after vaccination, even in the presence of maternal immunity. Early immunity is necessary to counter massive early exposure in the field. Vaccination protects against pathologic and immunosuppressive responses but not against infection or shedding. The efficacy of different MD vaccines varies but in practice vaccination is uncommonly effective, providing more than 95% protection in the field. Serotype 2 and 3 vaccine strains interact synergistically. Host genetic factors influence vaccine efficacy and involve interactions between Mhc alleles and vaccine serotypes. Some chickens develop MD at older ages for reasons that may involve immunosuppressive stress. The widespread use of vaccines has resulted in the emergence of highly virulent strains, which is one of the major challenges to long term control of this disease. Efforts to counter this problem are divided between the quest for more effective vaccines and strategies to slow the pace of evolutionary change.

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13 Control of Bovine Herpesvirus Diseases

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Bovine herpesvirus has continued to induce significant economic losses following respiratory or reproductive infection. Following infection of the respiratory tract, the virus significantly enhances the animal's chance of developing secondary bacterial infection with subsequent pneumonia and death if not treated with antibiotics. This respiratory disease complex has been estimated to cost the North American cattle industry between \$0.5-1 billion annually. For these reasons, producers have been interested in either vaccinating animals to reduce infection or eliminating the infection completely. Approaches to reducing infection include vaccinating animals. Most of the currently licensed vaccines are either killed or live attenuated vaccines produced by conventional technology. Another approach includes serological testing to eliminate seropositive animals. This approach is currently used in artificial insemination units as well as in some countries where serological testing and slaughter have been used to eradicate disease from that country. Eradication of the virus from a country is possible using serological testing and slaughter if the level of infection is low. However, if the rate of infection is high, alternative approaches must be developed. One such approach includes developing a marker vaccine in parallel with a diagnostic test. The recent licensure of a gE defective mutant is currently being used in some European countries to eradicate BHV-1. Other marker-based vaccines including subunits, DNA vaccines, or live vectored vaccines being developed are based on a similar principal. The types of vaccines currently in use with their advantages and disadvantages will be presented.

15 Antigen Presentation by CD1

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We have found that the universe of lipid antigens can stimulate specific T cell responses that are mediated by CD1 antigen presenting molecules. This is possible because the CD1 antigen presenting elements contain hydrophobic antigen-binding pockets that bind the lipid tails of antigens, rather than peptide binding grooves like MHC molecules. CD1 restricted T cells that are cytotoxic and/or secrete IFN- γ are implicated in host defense. We are defining the foreign antigens presented by CD1, the pathway of processing and trafficking of CD1 molecules that allows them to survey intracellular compartments for microbial antigens and their role in humans and animal models of infectious diseases.

To examine the ability of lipid antigens presented by CD1 to serve as a new approach to vaccine development for microbial infection, we have developed a mycobacterial lipid based vaccine. Immunization of guinea pigs revealed that lipid vaccinated animals had moderately reduced CFU in the lung and markedly improved histopathologic changes in the lung compared with vehicle immunized animals. Granulomas were markedly smaller and had little central necrosis. Lipid vaccinated animals also revealed less weight loss during the 5 week post infection period than did vehicle vaccinated animals.

14 Role of CpG Motifs in DNA Vaccines

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Role of CpG Motifs in DNA Vaccines

CpG motifs are unmethylated cytosine-guanosine dinucleotides within the context of certain flanking sequences. Such motifs, which are commonly found in bacterial DNA, have wide-ranging immune stimulatory effects on both innate and adaptive immunity. It has been demonstrated in animal models that synthetic oligonucleotides containing CpG motifs (CpG ODN) are potent Th1 vaccine adjuvants that can enhance both humoral and cell-mediated antigen-specific responses against when administered together with a wide variety of antigens. This has also been shown in humans with a hepatitis B vaccine. Plasmid DNA, by virtue of being synthesized in bacteria, have numerous unmethylated CpG, many of which have the potential to be immunostimulatory, although these could be further optimized for immune stimulatory effects. In addition, there are other sequences that can actually neutralize the stimulatory effects of the CpG motifs. We first attempted to improve the immunogenicity of DNA vaccines by mixing the antigen-expressing plasmid with CpG ODN, however the phosphorothioate backbone of the ODN appeared to out-compete the plasmid DNA for cell surface binding sites and prevented transfection, and hence antigen expression. We next tried removing many of the neutralizing motifs by site-directed mutagenesis and cloning in different numbers of CpG motifs. Both of these steps improved immunogenicity, however if too many CpG motifs were added, the enhanced immunogenicity of the vaccine was lost, possibly due to plasmid instability with its increased size, and/or down-regulation of the CMV promoter by the cytokines (e.g., type I and II IFNs) induced by the CpG motifs.

16 The Role of Toll-Like Receptors in Orchestrating the Innate Immune Response

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Macrophages represent one of the cornerstones of the innate immune system. They detect infectious organisms via a plethora of receptors, they phagocytose them, and then orchestrate an appropriate host response to them. While the inflammatory pathways leading to appropriate host response have been reasonably well defined, it has been unclear how macrophages are able to define the threat precisely; how does the cell know, for example, whether the vacuole within it contains a Gram-positive or Gram-negative bacterium? Recent work from a number of laboratories indicates that the Toll-like receptors play a key role in reading the bar code of invading microorganisms and act as adjuvant receptors. The mechanism by which they do this and how this is coupled to the phagocytic response will be discussed.

1. Underhill, D.M., A. Ozinsky, A.M. Hajjar, A. Stevens, C.B. Wilson, M. Bassetti, A. Aderem. 1999. The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens. *Nature* 401:811-815.
2. Aderem, A. and R.J. Ulevitch. 2000. Toll-like receptors in the induction of the innate immune response. *Nature* 406:782-787.
3. Aderem, A. and D.A. Hume. 2000. How do you see CG? *Cell* 103:993-996.
4. Hayashi, F., Underhill, D.M., Ozinsky, A., Smith, K.D., Yi, E. C., Goodlett, D. R., Eng, J. K., Aderem, A. 2001. The immune response to bacterial flagellin is mediated by Toll-like receptor-5. *Nature* 410(6832): 1099-103.

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17 Heat Shock Proteins as Adjuvants

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Our recent observations support a critical role for HSPs in cross-priming or indirect presentation. We have shown previously that (see 1 for review):

Homogeneous preparations of HSPs gp96, calreticulin, hsp 90 or hsp70 are associated with peptides derived from cellular proteins, incl. normal self proteins or mutated or foreign proteins.

If HSPs (which are actually HSP-peptide complexes) are injected into immunocompetent hosts, the hosts develop potent antigen-specific CD8+ and CD4+ T cells. This response is directed at the altered or foreign peptides and not against self peptides not against the HSPs themselves. The mechanism of immunogenicity of HSP-peptide complexes is clear and involves the interaction of the HSPs with macrophage or dendritic cells through the CD91 HSP receptors, followed by re-presentation of the HSP-chaperoned peptides by the MHC I and MHC II molecules of the macrophage/dendritic cells. We and others have also shown that the HSP-associated peptides from a given cell are not limited to those that may bind the MHC I alleles of those cells and that HSP-peptide complexes derived from cells may be used to cross-prime (2,3). We shall show data that indicate that not only can HSP-peptide complexes cross-prime, but that they are essential for cross-priming (4).

19 Rotavirus Vaccine Development by the Global Alliance for Vaccines and Immunization

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Rotavirus is the most common cause of severe diarrhea among children worldwide and a top priority for new vaccine development. An agenda of priorities has been prepared to speed development, testing and introduction of rotavirus vaccines into routine programs for childhood immunization in developing countries. First, a number of epidemiologic activities are needed to make pediatricians and health leaders aware of the burden of rotavirus diarrhea in their own countries and knowledgeable and willing to embrace rotavirus vaccines when these become licenced. Second, donor assistance will be required to test vaccines in developing countries. For the multinationals, third world markets are not their first order priorities so donor investments could permit simultaneous rather than sequential testing of new rotavirus vaccines making them available at the same time to a global market. For national producers, financial incentives can help manufacturers with experience in making other live oral vaccines redirect their efforts toward production of live oral rotavirus vaccines. Finally, support for activities of rotavirus vaccine advocacy, financing, and demonstration projects will involve many players in the international vaccine field. The goal of these activities if successful could be the prevention within a few years of the 600,000 rotavirus deaths each year and the one third of diarrhea hospitalizations for which rotavirus is the etiology. Given the sound scientific basis and extensive experience with live oral vaccines, efforts by both multinational and national producers need to be encouraged to speed up the final testing and introduction of these life-saving products.

18 Overview of the Research and Development Activities of the Global Alliance for Vaccines and Immunization

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The Global Alliance for Vaccines and Immunization (GAVI) addresses "access", "equity" and "investment" gaps in immunizing the world's children against vaccine-preventable diseases. GAVI's Task Force on Research and Development (TFR&D) selected live oral rotavirus vaccines, 9- and 11-valent pneumococcal conjugate vaccines, and conjugate vaccines to prevent meningococcal A disease as three vaccine projects to be accelerated for development and introduction into developing countries within 7 years. Through an interactive process with the global community involved in research on rotavirus and pneumococcus vaccines, focused "global research agendas" were prepared that identify critical research activities (e.g., assessments of disease burden; clinical trials in developing countries, etc.) that must be completed to provide an evidence base for subsequent decision making; the research agendas include timelines and estimated costs to complete the activities. In the future, immunization in developing countries would be more efficient and economical if all vaccines could be administered by non-parenteral (mucosal or transcutaneous) routes, require < 2 doses to elicit protection, capable of immunizing infants < 3 months of age, combinable with other vaccines, and formulated to resist extreme heat and cold (thereby diminishing dependency on the cold chain). Accordingly, to enhance the efficiency and simplicity of immunization services, the TFR&D is also selecting an array of "vaccine technologies" for accelerated development to: decrease dependence upon and ultimately eliminate the cold chain; improve tools to measure immunization services performance; reduce infectious wastes and ultimately eliminate the use of sharps (needles and syringes).

20 The GAVI Pneumococcal Vaccine Accelerated Development and Introduction Plan

O. Levine

Respiratory Diseases Branch, NIAID/CDC, Bethesda, MD

In 2000 the Global Alliance for Vaccines and Immunization (GAVI) set as an official objective "to accelerate the development, access, and use of pneumococcal conjugate vaccines in developing countries". This vaccine target was selected because of its high global disease burden and because the vaccines were considered 'low hanging fruit', i.e., they had a high likelihood of being available for use by 2007.

To achieve this objective, an Accelerated Development and Introduction Plan (ADIP) was developed through a GAVI-sponsored process of input from a broad range of constituencies (e.g., academia, industry, bilaterals, multilaterals, foundations, NGOs, and others from developing and industrialized countries). The process started with a meeting convened by the Task Force on Research and Development aimed at defining priority research and development activities that would contribute to meeting the GAVI objective. Most recently, McKinsey and Co., a global strategic consulting firm, has participated in an attempt to develop the ADIP as a framework for a public-private partnership to assure access to an affordable and adequate supply of pneumococcal conjugate vaccine for developing countries as early as 2006.

The ADIP is a target-driven plan with measurable objectives for uptake in each year. The plan's activities are designed to achieve these targets by: 1) establishing the value of pneumococcal vaccination (by showing disease burden and vaccine safety and effectiveness); 2) communicating the value of the vaccine (by generating evidence-based advocacy and demand for vaccination); and 3) delivering value (by assuring an affordable price, an adequate supply, and credible financing).

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21 The Meningitis Vaccine Project: A Partnership Between WHO and PATH F. Marc LaForce, M.D.

Over the last 100 years, large meningitis epidemics have caused enormous suffering in Sub-Saharan Africa. These outbreaks occur every 8 to 12 years with high attack rates and high mortality. Control of these epidemics using polysaccharide vaccines has been difficult and African public health officials would welcome the development of conjugate meningococcal vaccines. In fact, conjugate meningococcal vaccines were studied in Africa in the early 90s but were not pursued because they were viewed as risky and unlikely to be profitable. The 1996 African meningitis epidemic resulted in 188,000 reported cases. This disaster re-engaged public health officials and donors to search for a solution to this problem. In June 2001 the Meningitis Vaccine Project (MVP), a partnership between the Program for Appropriate Technology in Health (PATH) and the World Health Organization (WHO) was created through core funding from the Bill & Melinda Gates Foundation to help address the important public health problems caused by meningococcal disease in Sub-Saharan Africa. The principal goal of the project was to develop, test, license and widely distribute meningococcal conjugate vaccines appropriate for Africa. In addition there is a mandate that the vaccines be affordable and supplied in sufficient volume to achieve public health impact. Two vaccines are being developed: one is a polyvalent EPI vaccine (DTP,HepB, HiB, conjugate A/C) and a second A or A/W135 conjugate vaccine for mass immunization campaigns for ages 1-29. The recent upsurge in meningococcal meningitis due to W135 in Burkina Faso has added new pressure for the expeditious development of these products.

23 Pathogenesis of HPV-Associated Cancers K. V. Shah Molecular Microbiology & Immunology, Johns Hopkins University, Baltimore, MD

Over the past few years, HPV infections have been linked etiologically to squamous cell carcinoma and adenocarcinoma of the cervix, some cancers at other lower genital tract sites, and to a subset of cancers of the oropharynx. HPV infections appear to account for all cases of cervical cancers worldwide, in rich nations and in poor nations, and are regarded as a 'necessary event' for the development of cervical cancer. In contrast, vulvar cancers are etiologically heterogeneous. HPVs are linked to vulvar cancers with basaloid pathology, occurring in younger women, and preceded by vulvar intra-epithelial neoplasia. The more common keratinizing vulvar cancers of older women are unrelated to HPV infections. Head and neck cancers (cancers of the oral cavity, pharynx and larynx) comprise cancers of probably many diverse etiologies. The subset of cancers linked to HPV infections is located predominantly in the oropharynx (tonsils, base of tongue, soft palate), frequently has a basaloid pathology, and has a better prognosis and less frequent P53 and PRb mutations than HPV-negative oropharyngeal cancers. The strong tropism of HPVs for epithelial tissue as well as the lack of a viremic phase in HPV infections account, in part, for the locations of the HPV-associated cancers. The HPV-based immunotherapeutic vaccines which are being developed for cervical cancer will probably be also effective for treatment of HPV-associated cancers and pre-cancers at other sites. In addition, the prophylactic vaccines will protect against HPV-associated cancers not only by stimulating immunity in the immunized individuals, but also by diminishing viral transmission in the community.

22 Vaccine Technology Developments of the Global Alliance for Vaccines and Immunisation G. Dougan Centre for Molecular Microbiology and Infection, Imperial College of Science, Technology and Medicine, London, UNITED KINGDOM

If we are ever to achieve complete vaccine coverage against common diseases in all areas of the world new technologies will have to be used to improve our existing range of licensed vaccines. Many licensed vaccines are not ideal for large-scale vaccination programmes, particularly in developing countries. Factors such as expense, number of doses, sensitivity to freezing/heating etc. inhibit their effectiveness and generate wastage. Further, vaccines that require injection run the risk of spreading infection if the syringes are not disposable. The Global Alliance for Vaccines and Immunisation (GAVI) has undertaken, through a team of experts, a review of existing technologies that could be potentially applied to improve the delivery and efficacy of vaccines in the field. This review has been conducted over a period of several months. The outcome and some conclusions from this review will be discussed.

24 Endpoints for Preventive Human Papillomavirus (HPV) Vaccine Efficacy Trials R. D. Pratt, K. L. Goldenthal FDA, Rockville, MD.

This presentation will cover endpoints proposed for clinical efficacy trials to evaluate preventive HPV vaccines containing "oncogenic" HPV types. The ultimate goal for these vaccines is the prevention of cervical cancer. Since infection with an oncogenic HPV type is thought to be a necessary step in the pathogenesis of most cervical cancer, a number of potential endpoints proceeding from the initial infection are considered. Endpoints in clinical studies may be defined as measurable outcome variables following an experimental intervention. Primary efficacy endpoints are usually selected to provide an outcome measure of the greatest clinical relevance. In general, in efficacy trials of vaccines, prevention of a disease is commonly used as the primary outcome variable. However, the severity and stages of disease can vary considerably, and endpoints based on preventing disease of greater or lesser severity may be appropriate. The following endpoints have been considered for preventive HPV vaccines: (a) Incident HPV infection, (b) Persistent HPV infection by oncogenic HPV types, using various definitions of persistent, (c) Endpoints based on cytology, in association with oncogenic HPV types, (d) CIN 1 histology, adenocarcinoma in situ (AIS) of the cervix, or worse, in association with oncogenic HPV types, (e) CIN 2/3 histology, adenocarcinoma in situ (AIS) of the cervix, or worse, in association with oncogenic HPV types, and (f) Cervical cancer (invasive). Most of the interest has been focused on (b) and (e), above. The potential application of the FDA accelerated approval regulations to this situation can also be considered.

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Trials of Preventive Vaccines

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Prophylactic HPV vaccines to prevent the HPV infections that cause cervical cancer and its precursor lesions are under active development. In NCI sponsored clinical trials of a non-infectious HPV16 L1 virus-like particle (VLP) vaccine, low dose intramuscular injection of VLPs induced high titers of virion neutralizing serum IgG, without substantial side effects, in normal volunteers. Adjuvant was not required to induce high and persisting titers.

Cervical secretions of vaccinated women also contained substantial titers of VLP specific IgG, in general, approximately one tenth the levels detected in serum. However, serum titers were not always predictive of cervical titers for individual women, suggesting individual variation in the capacity to transudate serum IgG. In women with a normal menstrual cycle, there was a 10-fold drop in both total and VLPs specific cervical IgG around the time of ovulation. The cervical antibody titers were more uniform across the contraceptive cycle in women taking oral contraceptives.

A mucosally delivered VLP-based vaccine could potentially induce secretory IgA, in addition to systemic IgG, and it could potentially be more easily administered to preteens in a non-clinical setting. There is mounting evidence from animal studies, and from preliminary studies in women, that mucosal delivery of a VLP-based vaccine may be a viable option to intramuscular injection. However, the consistency and efficiency of VLP vaccination by a mucosal route will likely need to be improved to make this a practical alternative.

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Status of Prophylactic Vaccines Against Cervical Cancer

K. U. Jansen

Vaccine Microbial Research, Merck & Co., Inc., West Point, PA

Human papillomaviruses (HPVs) infect cutaneous, genital and respiratory epithelia in a tissue-specific manner. Infection with HPVs is widespread throughout the world, and viral infection is closely associated with both benign and malignant lesions. The overall percentage of HPV infection (either current or previously encountered) in a population could be as high as 75%. There are now over 100 HPV types described; from a public health standpoint however, only a small number of these different HPV types cause the majority of clinically important diseases. HPV16 and 18 are strongly associated with high-grade anogenital lesions and invasive cancers and are found in ~70% of all cervical squamous cell carcinomas and ~90% of adenocarcinomas. The causal link of HPV and cervical cancer has been clearly established both from population based studies as well as animal models. Despite the existence of good screening programs for precursor lesions of cervical cancer in the US, there are still ~14,000 cases diagnosed each year and ~5,000 women will die from the disease and screening is expensive. In developing countries where access to routine cervical cytological screening is nonexistent or difficult, cervical cancer is the most common malignancy with an estimate of ~500,000 new cases and ~200,000 associated deaths annually. Therefore, an effective and safe prophylactic vaccine would be highly desirable. Approaches to vaccine design and data from early phase prophylactic clinical trials will be discussed.

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Breeding New Vaccines

R. J. Howard

Maxygen, Inc., Redwood City, CA

MolecularBreeding[®] allows the rapid generation of recombinants of genes encoding protein antigens, promoter elements, vector backbones, protein immunomodulators and protein adjuvants. A set of parental genes are identified from Nature and used as the initial breeding stock. The library of variants from MolecularBreeding[®] is screened or selected to identify those recombinants with a phenotype superior to all of the parental genes. The several progeny so derived are then used as the parents of additional rounds of MolecularBreeding[®] where the criteria for success become more demanding at each round until the required set of properties for vaccine development are captured in the progeny genes. DNA vaccines have been proven repeatedly successful in laboratory animals. This vaccine modality is perceived to offer numerous advantages for the rapid discovery and manufacture of vaccines for diverse diseases, with the potential to address longstanding vaccine discovery and development opportunities in the tropics that are otherwise neglected for commercial consideration. Clinical trials are underway at numerous laboratories worldwide with early indications that additional vector development and delivery improvements are essential before general applicability of this modality. Maxygen is using MolecularBreeding[®] to develop improved antigens and vectors targeted to diverse infectious diseases of significance to developing nations. Maxygen has applied this technology platform to generate novel components of DNA vaccines and recombinant protein vaccines. The technology is also being used to develop novel animal models for vaccine testing and to increase the yield and quality of vaccines in the manufacturing process. Several examples will be described.

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29 A Novel Viral Vector for Vaccination H. C. J. Ertl, J. Fitzgerald The Wistar Institute, Philadelphia, PA

E1-deleted human serotype 5 adenoviral recombinants induce unsurpassed T and B cell responses to the inserted transgene product including antigens of HIV-1. Most humans are exposed to common human adenovirus serotypes, including serotype 5, periodically from childhood onward. The ensuing serotype-specific virus-neutralizing antibodies would likely impair immune responses to the transgene product of the corresponding serotype adenoviral recombinant vaccines. To circumvent this anticipated interference we developed an adenoviral recombinant using the chimpanzee adenovirus 68 (C68). C68 does not circulate in the human population and antibodies to human serotypes fail to neutralize C68, thus providing an improved alternative to human serotype adenoviral vaccines to HIV-1. Vaccines based on E1-deleted recombinants of Adhu5 or AdC68 virus expressing a codon-modified, truncated sequence of HIV-1 gag p37 (termed Adhu5gag37 and AdC68gag37) were tested for induction of CD8+ T cell responses in Balb/c mice. Both recombinants induced gag-specific CD8+ T cell responses in Balb/c mice at surprisingly high frequencies during the acute effector and memory phases. The AdC68gag37 construct induced superior gag-specific CD8+ T cell activity. Priming or boosting with a heterologous vaccine carrier (vaccinia gag recombinant virus) augmented this response. Mice pre-exposed to Adhu5 failed to respond to subsequent vaccination with the Adhu5gag37 construct. In contrast, the gag-specific CD8+ T cell response was only slightly reduced in Adhu5-immune mice vaccinated with the AdC68gag37 vaccine. Both the Adhu5 and AdC68 recombinants infect immature dendritic cells driving their maturation indicated by phenotypic changes and cytokine release, with the AdC68 recombinants showing a greater effect.

31 Regulatory Issues for New Vaccine Technologies J. R. Daugherty, Ph.D. Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, MD

The Office of Vaccines Research and Review (OVR) is responsible for regulatory review of new Investigational New Drug (IND) Applications and Biologics License Applications (BLAs) for preventive vaccines and certain therapeutic vaccines. Through this review process, OVR ensures that both preventive vaccines and therapeutic vaccines for infectious disease indications are safe, effective, pure and potent, as specified in the Code of Federal Regulations (21 CFR 610). This presentation will focus predominantly on the preclinical testing of preventive vaccine candidates, and will first focus on the general regulatory issues that apply to preclinical testing in common to all vaccines. The second part of the discussion will cover regulatory issues specific to preclinical testing of new vaccine technologies.

30 Vaccine Development in Transgenic Animals H. M. Meade GTC, Framingham, MA

Production of sufficient quantities of properly folded antigens remains a challenge for the development of candidate vaccines. The C-terminal 42 kDa of the Plasmodium (P.) falciparum Merozoite Surface Protein1 (MSP-142) is one of the leading candidate antigens for a malaria vaccine. We used an innovative approach to produce this protein at high levels in the milk of transgenic mice. Initially, it was determined that the P. falciparum MSP142 gene of the FVO strain did not yield detectable protein using standard expression vectors to transfect COS cells or in the milk of transgenic mice carrying the gene under control of a strong milk specific promoter. Since no specific RNA could be detected in either system, we hypothesized this was due to the RNA instability motifs within the coding sequence as a result of the high AT content of the sequence, (76%). We therefore synthesized a version of the MSP-142 gene in which these sequences were replaced and the GC content increased to 50%. When transfected into COS cells, this version was expressed and resulted in expression of the protein into the media. Furthermore, transgenic mice carrying the synthetic MSP142 gene under control of the milk specific promoter, goat beta casein, secreted high levels of the MSP-142 protein into their milk. A non-glycosylated version of the MSP142 protein was also produced in mice. This strategy allowed the purification of sufficient quantities of these proteins for pre-clinical testing in Aotus nancymai monkey challenge studies

32 Assessing Vaccine Safety in Pre-licensure Clinical Trials: An Industry Perspective W. C. Gruber Clinical Research, Wyeth Vaccines Research, Pearl River, NY

Perspectives about vaccine safety have changed over time. Historically, the highly visible mortality and morbidity of vaccine preventable illness has outweighed public concerns about real and perceived vaccine risks. As major infectious causes of morbidity are eliminated from the public eye, new vaccine development faces increasing demands for clinical trials to rule out rare vaccine related events. This presentation will address the impact of current perceptions of vaccine benefits and risks, challenges to assessing low risk events in clinical trials, and the need to balance caution with accountability for progress in new vaccine development.

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33 **Assessing Vaccine Safety in Pre-Licensure Clinical Trials: A Regulatory Perspective**
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Bethesda, MD

For licensure, vaccines must be both safe and effective for their intended use. Safety is defined as “relative freedom from harmful effect to persons affected directly or indirectly by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time.” Safety is relative; risk tolerance may be influenced by: risk of vaccine-preventable disease versus risk of vaccine-related adverse events, alternative treatments (e.g., OPV v. IPV), and target population. In determining size of the safety database for vaccines intended for routine childhood immunization, one should consider: size of U.S. birth cohort; intended population (primarily healthy infants and children); individual children may derive no benefit from vaccination; and state laws frequently mandate vaccination. Characteristics of the vaccine must be considered in designing safety monitoring. In phase 2, safety evaluation can provide data on common reactions and administration of study vaccine with other vaccines. Phase 3 studies are designed to evaluate less common reactions and may use simplified designs wherein a subset is actively monitored for common local and systemic reactions, but all individuals are followed for serious adverse events and other specified events (e.g., health care provider visits). Safety data from randomized, well-controlled trials are the most interpretable, because such trials reduce the possibility of bias and provide more reliable estimates of relative risks, especially for “background” adverse events. Monitoring for vaccine safety continues post-licensure, and new adverse events may be identified as a vaccine is administered to a much larger population.

35 **Contentious Ethical Issues in Performing Clinical Vaccine Trials in Developing Countries: A Bioethical Perspective**
C. Weijer
Department of Bioethics, Dalhousie University, Halifax, NS,
CANADA.

The last two years have witnessed significant changes in the international regulation of research. In 2000, the *Declaration of Helsinki* underwent an important revision, and UNAIDS released the document *Ethical Considerations in HIV Preventive Vaccine Research*. In 2002, the Council for International Organizations of Medical Sciences (CIOMS) is bringing to a close the revision process for the *CIOMS International Ethics Guidelines*. Together these new ethical guidelines have important implications for the conduct of clinical vaccine trials in developing countries. First, early phase vaccine trials may now be conducted in developing countries, especially if parallel trials are conducted in the sponsor country. Second, sponsors have an obligation, where possible, to ensure that study participants have reasonable access to novel therapies upon completion of the trial. It remains unclear whether this requirement extends only to actual trial participants or the broader communities to which they belong. Third, it remains controversial whether the lack of availability of a vaccine in a host country due to cost or short supply legitimates the use of a placebo control.

34 **Vaccine Trials and the Assessment of Herd Immunity**
P. E. M. Fine
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Herd immunity implies indirect protection of non-immunes attributable to the presence of immune individuals in a population, and reflects reduction in infection transmission in and by vaccinated individuals. A decline in incidence by a factor greater than the product of vaccine uptake and efficacy indicates a combination of direct and indirect protection. It is possible to measure the magnitude of indirect protection in trials and in observational studies, by comparing incidence rates among non-vaccinees between populations with different levels of vaccine coverage. Formal trials to assess indirect protection raise a variety of problems relating to comparability and statistical power, and few have been carried out to date. Furthermore, indirect effects are a function of contact patterns within communities, and are thus likely to be less generalizable than are estimates of the direct effect of vaccines in protecting against disease, which are dependent upon a vaccinee’s immune response rather than upon the social fabric. Though indirect protection should increase the benefit/cost ratio of a vaccine, an individual who is protected only indirectly is dependent upon social context for this risk reduction, and may still contract the infection later in time. Thus the benefits of indirect protection depend upon coverage, and the relationship between age and severity. If severity declines with age, as with pertussis, indirect protection is likely to be beneficial; but if it increases with age, as with rubella, then it can lead to increased morbidity. Neither the measurement nor the evaluation of “herd immunity” is straightforward.