Vaccines Against Bioterrorism
P. K. Russell
Office of Public Health Preparedness, Department of Health and Human Services, Washington, DC

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.

Tuberculosis: Progress in the Development of a New Vaccine Against the Captain of All the Men of Death
M. A. Horwitz
Medicine, UCLA, Los Angeles, CA

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.

B Cell Memory and the Persistence of Antibody Responses
I.C. M. MacLennan
MRC Centre for Immune Regulation, University of Birmingham, Birmingham, UNITED KINGDOM

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.
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 capular 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 infection 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.

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 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.
The Foot-and-Mouth Disease Epidemic in the United Kingdom, 2001: Why Vaccination was Not Employed
A. I. DONALDSON
Div of epidemiology, INSTITUTE FOR ANIMAL HEALTH, Woking 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.

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Novel Strategies to Control Foot-and-Mouth Disease
M. J. Grubman, J. Chinsangaram, M. P. Moraes
USDA, ARS, NAA, Plum Island Animal Disease Center, 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 un inoculated animals, and swine given a single high dose of Ad5-FMDV A24 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 strategies suggest that a combination strategy can be successfully used in FMD-free countries to induce immediate and long-term protection.

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Vaccination Against Respiratory Diseases of Poultry: Avian Influenza and Newcastle Diseases
D. E. Swayne
ARS, Southeast Poultry Research Laboratory, USDA, Athens, GA

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 respiratory-like disease 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.

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 amnionic sac of 18-day embryos. Immunology 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 commonly 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.
### Antigen Presentation by CD1

**M. B. Brenner, M. Sugita, C. Dascher, X. Xiao, M. Vincent**

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 secret IFN-γ are implicated in host defense. We are defining the foreign antigens that are 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.

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

**A. Aderem**

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.


### Role of CpG Motifs in DNA Vaccines

**H. L. Davis**

Coley Pharmaceutical Group, Ottawa, ON, CANADA

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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.
Abstracts of Invited Presentations

17 Heat Shock Proteins as Adjuvants
S. Basu
Center for Immunotherapy of Cancer and Infectious Diseases, University of Connecticut Health Center, Farmington, CT

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 gsp96, 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).

18 Overview of the Research and Development Activities of the Global Alliance for Vaccines and Immunization
M. M. Levine
Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, MD

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 withstand 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).

19 Rotavirus Vaccine Development by the Global Alliance for Vaccines and Immunization
R. I. Glass, U. Parashar, J. Breshe, J. Gentsch
Viral Gastroenteritis Section, CDC, Atlanta, GA

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.

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, multilateral, 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 by 2010.

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).
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 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 basoloid pathology, and has a better prognosis and less frequent P53 and PRb mutations than HPV-negative oropharyngeal cancers. The strong tropism of HPV’s 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.

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 Th(rough) 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.
Trials of Preventive Vaccines
J. T. Schiller\(^1\), D. Nordelli-Haefliger\(^2\), D. R. Lowy\(^3\)
\(^1\)Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, MD, \(^2\)Centre Hospitalier Universitaire Vaudois, Lausanne, SWITZERLAND, \(^3\)Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, MD

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.
29 A Novel Viral Vector for Vaccination
H. C. J. Errlt, 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.

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.

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 (OVRR) 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, OVRR 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.

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.
Assessing Vaccine Safety in Pre-Licensure Clinical Trials: A Regulatory Perspective
K. Midthun
Office of Vaccines Research and Review, CBER/FDA, 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.

Vaccine Trials and the Assessment of Herd Immunity
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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.

Contentious Ethical Issues in Performing Clinical Vaccine Trials in Developing Countries: A Bioethical Perspective
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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.
A Gene-based Vaccine Formulation is Safe, Immunogenic and Protective Against Human Respiratory Syncytial Virus (RSV) in Infant Rhesus Macaques
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Background: Currently, there is no vaccine for use against RSV, nor is there consensus regarding the most appropriate strategy to use in the neonatal population. DNA vaccines elicit humoral and cellular immunity, and are safe in neonatal animal models. We hypothesized that plasmid DNA encoding RSV proteins would induce humoral and cellular immunity that would be protective against RSV in infant rhesus monkeys.

Methods: Monkeys were immunized at 1.5 and 2.5 months. Eight weeks post-inoculation, vaccines and controls were challenged with human RSV. RSV-specific antibody was assayed using ELISA. Cellular responses were assessed using ELISPOT to measure IFNg production. Virus titers and RSV mRNA levels were determined in the lung. Pulmonary histopathology was also assessed. Mock-challenged controls were included.

Results: RSV-specific IgG was induced and remained elevated 4 months post-inoculation. Two non-responders had higher levels of maternal antibody pre-immunization. Non-responders could achieve comparable levels, 4 months post-inoculation. RSV-IgG was unchanged in controls prior to challenge. Cellular responses were greater in vaccinated infants, and this correlated to levels of RSV in the lung.

Conclusions: DNA vaccines can be immunogenic and protective in infant monkeys injected at 1.5 months. High levels of maternal antibodies can affect humoral responses to DNA vaccines, but do not prevent the induction of specific IgG following exposure to virus. Moreover, the presence of maternal antibody does not affect vaccine-induced cellular immunity, and the ability to clear virus.

Gene Gun Vaccination with a Structural Gene of Hepatitis E Virus Induces Protective Immunity Against Hepatitis E Virus Infection in Cynomolgus Macaques
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Hepatitis E is a major public health problem in several developing countries. Recombinant proteins encoded by full-length or truncated constructs of hepatitis E virus (HEV) open reading frame 2 (ORF2) have been shown to confer partial or full protection against HEV in experimentally infected cynomolgus macaques (cynos). A full-length HEV ORF2 (Burme strain) was used for genetic vaccination of cynos, which were subsequently challenged with a heterologous HEV strain (Mexico). Of the twelve cynos used in this study, 4 were vaccinated intramuscularly, 3 via gene gun and 2 intradermally with the HEV ORF2 DNA cassette. 3 control animals included one vaccinated intramuscularly and two via gene gun with a mock DNA construct. Highest levels of anti-HEV antibodies were detected in all the three animals vaccinated via gene gun while modest levels of anti-HEV antibodies were detected in four intramuscularly vaccinated cynos. None of the animals vaccinated intradermally or in the control group responded to vaccination. All cynos were subsequently challenged intravenously with a heterologous strain of HEV (Mexico strain). All the three animals vaccinated via gene gun and two of the four intramuscularly HEV DNA vaccinated-cynos were protected against HEV infection. These findings demonstrate the feasibility of DNA vaccination for the protection of HEV infection in experimentally infected cynos and emphasize the significance of route of vaccination for inducing a potent immune response by DNA vaccination.

Recombinant SV40 Vaccine Delivery Vehicles Encoding HIV-1 gp120 and IL-12 Elicit Strong Cell-mediated Immune Responses Against gp120
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Background: In attempting to develop a vaccine against HIV, some recent studies report that several cytokines, e.g. IL-2 and IFN-g, improve immune responses against lentivirus antigens. Since IL-12 promotes adaptive immunity, and is effective at much lower concentrations than IL-2, we incorporated it into an anti-HIV vaccine strategy. We investigated whether recombinant SV40 vectors (rSV40) carrying murine IL-12 (SV(mIL-12)) enhance immunity against gp120 in mice co-immunized with rSV40 encoding HIV-1NL4-3 gp120 (SV(gp120)).

Methods: Mice received SV(gp120) +/- SV(mIL-12) monthly. Cloned gp120-expressing cells were used as targets in a cell-based ELISA (CELSIA) to measure antibody responses against gp120. The same cells were targets in 51Cr-release assays to measure gp120-specific cytotoxic responses.

Results: Single inoculation of gp120 +/- IL-12 did not elicit detectable antibody against gp120. However, immunization with SV(gp120) alone, or SV(gp120) plus SV(mIL-12) before or after SV(gp120) elicited very high levels of specific cytotoxicity (> 60% specific lysis). SV(mIL-12) alone induced no detectable cytolytic response against gp120.

Conclusions: A single treatment of gp120 +/- IL-12 did not elicit detectable humoral immunity against gp120-expressing targets. However, specific anti-gp120 cytolytic lymphocyte responses were very high in mice following 2 immunizations with SV(gp120) or combined with separately administered SV(mIL-12). These results suggest that immunostimulatory cytokines like IL-12 may enhance immune responses generated using rSV40 vectors encoding lentiviral antigens.

Direct Linkage of Immunostimulatory DNA to a Variety of Proteins Dramatically Enhances Th1 and CTL Responses
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Background: Immunostimulatory DNA sequences (ISS) stimulate cytokine production, increase antigen presentation, and promote the development of Th1 and CTL in the presence of antigen. Direct linkage of ISS to proteins dramatically enhances Th1 and CTL responses compared to proteins simply mixed with ISS.

Methods: An ISS oligonucleotide was chemically linked to HIV p24, hepatitis B surface antigen, and the ragweed allergen Amb a 1. Mice were immunized with the ISS-linked proteins, proteins alone or with proteins mixed with ISS. Antibody responses against the proteins were measured by ELISA. Protein-specific in vitro cytokine responses were measured by ELISA and by ELISPOT. CTL responses were measured by 51Cr release assay.

Results: ISS-linked Amb a 1 induced significantly higher antigen-specific IgG2a and IFNg responses than allergen alone or allergen mixed with ISS. ISS-linked p24 showed the same IgG2a and IFNg enhancement, and additionally induced CTL responses significantly higher than antigen alone or p24 mixed with ISS. ISS-linked HBsAg also enhanced IFNg and CTL responses, but induced very low HBsAg-specific antibody responses, perhaps due to blocking of B-cell epitopes by the conjugation procedure. IgG2a antibody response was restored and enhanced by co-injecting unmodified HBsAg and the ISS-linked antigen.

Conclusions: Linking ISS directly to antigens and allergens is a broadly applicable method for enhancing Th1 and CTL responses. Proteins linked to ISS induce much stronger T cell responses than proteins mixed with ISS.
S5 Predominance of IgG1 Against Hepatitis C by a Combination of DNA Plasmid, Core E1/E2 Protein and Mountanide
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Mice were immunized intramuscularly with a candidate hepatitis C virus vaccine at week 0, 4 and 8 with 100 mg DNA plasmid and/or 25 mg recombinant Core E1/E2 polyprotein and 50 ml Mountanide ISA-51 as an adjuvant. Four weeks after the last injection, all groups of mice were sacrificed and blood samples were collected. Levels of specific Hepatitis C antibodies (IgG, IgG1 and IgG2a) were measured by ELISA. Six groups of mice were immunized, including a negative control group consisting of expression plasmid with no HCV insert (group A), recombinant (R) plasmid alone (group B), R plasmid priming and boosting twice with protein plus mountanide (group C), R plasmid plus recombinant protein and adjuvant priming and boosting twice with the same preparation (group D), R plasmid with adjuvant priming followed by two boosters with R plasmid and adjuvant (group E), the last group consisted of protein plus adjuvant priming followed by two booster immunizations with protein and adjuvant (group F). Only groups D (OD[average]=0.90±0.30±SD), p<0.01) and F (OD=0.40±SD, p<0.05) demonstrated a significant increase in the total IgG titer before and after immunization. IgG1 was the predominant antibody detected in group D (OD=0.22±0.17). IgG2a was not detected. The combination of an expression DNA plasmid, core E1 and E2 proteins and mountanide induce a high antibody titer with predominance of IgG1 antibodies.

S6 Enhanced Plasmid Vaccine Immune Potency Through Engineering Novel Costimulatory Molecules
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Background: Analysis of first generation HIV-1 plasmid vaccines in 167 individuals demonstrated that the vaccines were well tolerated and induced CD4 T cell responses, the CTL responses were below a 20% response rate requiring improvement.

M & R: To improve responses, we employed optimized constructs which exhibit 20-100 expression enhancement. However, additional enhancements are still required. We report enhancement of the CD8 response by engineering costimulatory molecules. We observed that B7.2 was more effective than B7.1 as a plasmid vaccine. We developed gene swaps between these molecules. Adjucy segregating not with the Ig like V region binding domains but was associated with the constant domain. We suspected that the C domain of CD80 was limiting the costimulatory activity so we removed this domain. This resulted in a dramatically enhanced immune response as measured by CTL or Elispot. In Biacore analysis these constructs bind with 14 fold lower activity to CTLA-4 providing a significant vaccine advantage. Furthermore we have tested these combinations with cytokines genes, particularly IL-15 which further enhances the T cell response.

Conclusions: These surprising results suggest an important clue into a previously unrecognized immune regulatory system of CD80/86 costimilation which has important implications for the design of vaccines for prophylaxis or therapy.

S7 Sequence Variation in the Influenza A Virus Nucleoprotein Associated with Escape from Cytotoxic T Lymphocytes
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CD8+ cytotoxic T lymphocytes (CTL) contribute to the control of viral infections by recognizing peptides of viral proteins presented by MHC class I molecules on infected cells. Some viruses have developed strategies to evade recognition by CTL. One of these strategies involves antigenic variation in CTL epitopes as described for viruses chronically infecting their host like EBV, HIV HBV and HCV. Here we show three examples of variation in CTL epitopes in the influenza virus nucleoprotein (NP) associated with escape from CTL immunity. The first two involve a mutation at position 384 of the NP, which is the anchor residue of a HLA-B27 restricted epitope NP383-391 (SRYWAIRTR) and the HLA-B8 restricted epitope NP 380-388 (ELRSRYWAI). It was shown that these mutations have arisen in 1993/1994 and that these mutant variants completely replaced the virus strains containing the wild type epitopes. Furthermore, T cell recognition was completely abrogated by the R384G mutation. A third example of variation in an influenza virus CTL epitope was found in a newly identified HLA-B35 restricted CTL epitope. This immunodominant epitope exhibited extensive amino acid sequence variation and the variants emerged in a chronological order. Again CTL specific for older variants failed to recognize more recent strains of influenza A virus, indicating an escape from CTL immunity. Thus, in addition to the introduction of mutations in the surface glycoproteins like the hemagglutinin, allowing escape from antibody mediated immunity, there is now evidence that influenza viruses can escape from CTL mediated immunity.

S8 Intranasal Delivery of Dry-powder Influenza Vaccine Induces Mucosal and Systemic Immune Responses in Rats
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Purpose: Influenza vaccines, in liquid form, have been widely reported to elicit both mucosal and systemic immune responses in animals when administered intranasally. Powder vaccine formulations offer advantages over liquids in their stability and ease of storage and transportation, enabling cost effective worldwide vaccine delivery. In this study, we demonstrated the effectiveness of intranasal dry-powder formulations of influenza vaccine in eliciting both mucosal and systemic immune responses.

Methods: Brown Norway rats were immunized intranasally with powder formulations containing inactivated whole influenza virus (A/PR/8/34 H1N1). Controls were immunized with a liquid formulation, both intranasally (IN) and intramuscularly (IM). Immunizations were given at 2-3 week intervals. Serum and vaginal samples were collected 2-3 days after each immunization and nasal lavage fluid was collected before the termination of the experiment. Antibody responses were measured by ELISA.

Results: Strong serum antibody responses were detected following IN dry-powder influenza vaccination. In this study, IN dry-powder vaccination elicited serum antibody responses at least as strong as IN or IM liquid vaccination. Strong nasal IgA responses were also observed in IN dry-powder vaccination groups, while the IM injection group remained negative. Weak IgA responses were also seen in vaginal samples of rats intranasally immunized with dry-powder vaccine.

Conclusions: Dry-powder formulations of whole inactivated influenza vaccine are promising candidates for intranasal vaccination. These vaccines can elicit strong systemic immune response and nasal mucosal immune response, and may elicit mucosal immune response in remote areas.
Nasal Proteosome-Flu vaccines Induce Enhanced Serum and Mucosal Antibodies in Previously Infected or Immunized Hosts

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Nasal (i.n.) proteosome vaccines, comprising nanoparticles of purified N. meningitidis outer membrane proteins co-formulated with antigens from target microorganisms, elicit potent mucosal and systemic immunity in animals and humans. Following successful pre-clinical studies with i.n. Proteosome subunit influenza vaccines (FluINsure), two Phase I clinical trials were performed. In humans, FluINsure was well tolerated and both single dose and two-dose regimens induced specific nasal IgA and serum HAI responses at levels associated with protection. We here report studies in mice investigating the benefits of FluINsure after prior exposure to influenza antigens or proteosomes in the form of either nasal proteosome vaccines, intramuscular split-flu vaccines, or live virus - conditions mimicking the potential immune status of humans receiving annual influenza vaccinations. Mice immunized nasally with FluINsure or live virus or injected with split-flu vaccine were re-immunized forty weeks later with FluINsure containing either homologous H1 or homotypic H1 variant influenza antigens. Such FluINsure re-immunizations boosted specific responses against the homologous or homotypic influenza strains, respectively, after primary immunizations with either nasal FluINsure or live virus, or intramuscular split-flu. In addition, while influenza responses were markedly enhanced by boosting with FluINsure, anti-proteosome Omp-specific serum IgG or nasal IgA levels remained minimal. These results support the concept that FluINsure containing homologous or homotypic variant influenza antigens would be appropriate in an annual influenza immunization program for persons previously immunized with nasal proteosome or injectable flu vaccines or who had been exposed to live influenza virus.

Genetic Stability of FluMist™, A Live Attenuated Influenza Virus Vaccine, Throughout the Manufacturing Processes

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FluMist® is a live attenuated influenza virus vaccine consisting of 6:2 reassortants with the six internal gene segments from an attenuated donor virus, and the HA and NA segments from the circulating wild-type (wt) virus. Due to the lack of proof-reading ability of the influenza virus-encoded RNA-dependent RNA polymerase, viral RNA replication often gives rise to RNA mis-incorporations which could potentially affect the attenuation phenotype. Thus, the genetic stability is of particular importance for live RNA virus vaccines during egg amplifications for mass production. This study reports the first detailed nucleotide sequence analyses using full-length genomic sequencing of FluMist products. Nucleotide consensus sequences of three vaccine strains, A/Beijing/262/95, A/Sydney/05/97, and B/Ann Arbor/01/94, were investigated throughout the manufacturing processes, including the Master Virus Seed (MVS), Manufacturer’s Working Virus Seed (MWVS), and the final Virus Harvest (VH). A total of 17 genomes including 13 manufacturing lots were analyzed. Consensus sequences of eight RNA segments of the three strains showed complete identity throughout their respective manufacturing stages. Moreover, sequence comparisons revealed a high degree of consistency between the vaccine strains and the attenuated donor virus with respect to the six internal gene segments. The predicted amino acid sequences of HA and NA segments remained unchanged between the vaccine strains and their corresponding progenitor wt viruses. These results demonstrate that FluMist® vaccine viruses are genetically stable during manufacturing process, and provide further assurance that FluMist® vaccine consistently maintains the attenuation phenotype as well as the desired antigenic properties of a live attenuated influenza vaccine.
S13 Mycobacterial Heat Shock Protein (Hsp) Fusions: A Novel Class of Immunotherapeutics for the Treatment of Human Papillomavirus (HPV) Infection
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Cell-mediated immunity (CMI) to HPV16 E7 (E7) is associated with the resolution of HPV-related lesions. An in-frame fusion protein comprised of mycobacterial Hsp65 and E7 (SGN-00101) induces CMI and tumor regression preclinically and provides clinical benefit in phase II immunotherapy trials of anogenital HPV diseases. To test other Hsp fusions, a series of constructs using different Hsp fused to E7 were tested for activity.

DNA constructs or recombinant proteins comprised of full-length Hsp10, Hsp40, Hsp65 or Hsp70 from M. tuberculosis fused with full-length HPV16 E7 were used (without adjuvant) to treat C57BL/6 mice with pre-established, E7-expressing TC-1 tumors. Primed splenocytes from mice immunized with Hsp fusion proteins were tested for cytolytic and cytokine-producing activity.

Tumor regression was observed in mice treated with pCMV/SGN-000101, but not pCMV/Hsp65, pCMV/E7, or pCMV/Hsp65 + pCMV/E7, confirming that the activity is a property intrinsic to the Hsp fusion. Treatment with SGN-000101, but not its constituent proteins, Hsp65, and E7, regresses tumors. Similarly, Hsp10-E7, Hsp40-E7 and E7-Hsp70 fusion protein treatment leads to tumor regression. Primed splenocytes from E7-Hsp70- or SGN-000101-immunized mice were cytolytic for E7-expressing targets and released IFN-g upon E7 restimulation. Immunization of mice with plasmids encoding Hsp-E7 fusion proteins, or Hsp-E7 fusion proteins, induces E7-specific CMI and regresses E7-expressing tumors. These results suggest that Hsp-E7 fusions are a potentially new class of immunotherapeutics for HPV-associated disease.

S14 Enhancing MHC Class I Antigen Presentation by Targeting Antigen to Centrosomes
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Background: Several strategies that increase proteasomal degradation of antigen have been shown to improve MHC class I presentation of antigen and enhance vaccine potency. We hypothesize that a strategy to target and concentrate antigen to centrosomal compartments may enhance MHC class I presentation of antigen and lead to improved vaccine potency.

Methods: We therefore created a novel fusion of g-tubulin, an established centrosomal marker, with a model antigen, human papillomavirus type 16 (HPV-16) E7, in a DNA vaccine, pcDNA3-tubulin/E7. DNA constructs of pcDNA3-E7, pcDNA3-tubulin, and pcDNA3 were also generated. These DNA vaccines were injected into mice using gene gun. The immunological response and anti-tumor effect of these vaccines were performed.

Results: The linkage of g-tubulin to E7 targeted and concentrated antigen to centrosomal compartments and resulted in enhanced MHC class I presentation of E7. These properties led to a dramatic increase in the number of E7-specific CD8+ T cell precursors in vaccinated C57BL/6 mice and a potent antitumor effect against E7-expressing tumors. In comparison, control constructs of wild-type E7, g-tubulin alone, or DNA plasmid without insert (pcDNA3) failed to enhance immune responses or antitumor effects in vaccinated mice. In addition, vaccination with pcDNA3-g-tubulin/E7 DNA in TAP-1-knockout and CD4-knockout mice revealed that the enhancement of E7-specific CD8+ T cell immune responses is TAP-1-dependent and CD4-independent.

Conclusion: Our data indicated that the potency of vaccines could be significantly improved by targeting antigen to the centrosome, which may have important applications for vaccine development.

S15 HLA-A2 and H-2Db-restricted CTL Activity Induced by SGN-00101—a Fusion Protein Comprised of Heat Shock Protein and Human Papillomavirus E7
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Infection with human papillomavirus type 16 (HPV16) is strongly associated with cervical carcinogenesis. It is believed that induction of T cell-mediated immunity, particularly cytolytic T lymphocytes (CTL), is important in eradication of HPV-induced lesions. Previous studies have shown that heat shock protein (Hsp) fusion proteins are capable of inducing potent antigen-specific CTL activity in experimental animals. In addition, the outgrowth of E7-expressing murine tumor cell line (TC-1) can be prevented by treatment with SGN-00101, an Hsp fusion protein comprised of Mycobacterium bovis BCG Hsp65 linked to HPV16 E7. In the present study, we investigated the ability of SGN-00101 to prime E7-specific CTL activity. Splenocytes from mice immunized with SGN-000101 were restimulated in vitro with HPV16 E7-derived CTL epitope peptides. CTL activities were measured using 51Cr-labeled peptide-pulsed or E7-expressing target cells. Results indicated that SGN-00101 was effective in priming HLA-A2-restricted CTL in A2.1/Kb transgenic mice and H-2Db restricted CTL in C57BL/6 mice. In addition to lysing target cells pulsed with peptides, the E7 specific CTL were capable of lysing target cells expressing E7 endogenously. Significant CTL activity was observed (60% at 100:1 E:T ratio) in both mouse strains. The present study supports the previous findings that Hsp fusion proteins are capable of inducing peptide-specific CTL activity. This study provides additional support for the use of SGN-00101 to treat HPV-related disease.

S16 Induction of HBcAg-Specific CTL Responses by a Heat Shock Protein Fused to the Core Antigen of the Hepatitis B Virus
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Individuals acutely infected with hepatitis B virus (HBV) typically recover from infection and exhibit strong, broadly specific CD8+ CTL responses, while chronically infected patients display relatively weak, narrowly focused responses. Resolution of chronic hepatitis may be induced by immunotherapy that primes effective HBV-specific CTL responses. Heat shock protein (Hsp) fusion proteins have been shown to prime potent CTL activity in experimental animals. We generated recombinant fusion proteins containing sequences from the HBV core antigen (HBc) and the 65 kDa Hsp from Mycobacterium bovis BCG. Spleen cells from mice immunized without exogenous adjuvant were restimulated with HBc-derived CTL epitope peptides. Cells from fusion protein-primed mice exhibited a high degree of lytic activity, whereas cells from mice immunized with HBc antigen alone displayed poor target cell lysis. The lytic activity was specific for multiple CTL epitopes, including an HLA-A2-restricted epitope. Effector cells lysed both peptide-pulsed target cells and also target cells transfected to express endogenous HBc antigen. The CTL released IFN-γ and TNF-α, cytokines thought to be involved in inhibition of hepatic viral gene expression. In comparison with control mice, Tlr4 deficient C57BL/10ScNcr mice responded relatively poorly to fusion protein immunization, indicating that CTL priming is dependent, at least in part, upon this toll-like receptor. The results of these experiments clearly demonstrate the potential value of Hsp fusion proteins in the immunotherapy of chronic HBV hepatitis.
Immunization of mice with pneumococcal surface adhesin (PsaA) emulsified in Complete Freund’s adjuvant (CFA) has been reported to provide protection against systemic infection with Streptococcus pneumoniae. Because the use of CFA is not acceptable in humans we sought to develop alternative means of enhancing the immunogenicity of protein antigens. We linked coding sequences for PsaA to sequences encoding either murine (m) interleukin (IL)-2, mIL-4, or two copies of an immunogenic nonapeptide derived from mL1-1b. The PsaA-cytokine constructs were cloned and expressed in E. coli. Mice immunized twice with PsaA-IL2, or PsaA-IL4 (and to a lesser extent, PsaA-IL1b) responded with PsaA specific antibody production comparable in magnitude to that of mice primed with PsaA in CFA and boosted with PsaA in incomplete Freund’s adjuvant (PsaA-Adj). Antibodies elicited by PsaA-Adj were predominantly of the Immunoglobulin (Ig) G1 sub-class while PsaA-IL2 and PsaA-IL4 elicited a substantial amount of IgG2a in addition to IgG1. Mice immunized with PsaA-Adj, or PsaA-IL4 were partially protected against intra-peritoneal challenge with virulent S. pneumoniae (30% survival beyond 15 days post-challenge). Mice immunized with PsaA without adjuvant, or PsaA-IL2 exhibited zero, or 5% survival rates, respectively, following challenge. In contrast mice immunized twice with capsular polysaccharide were 100% protected. The modest levels of protection in mice with serum antibodies to PsaA may be explained in part by the inaccessibility of antibody to PsaA on the surface of encapsulated S. pneumoniae.

Coccidioides immitis is a fungal pathogen of humans that resides in desert soil of Southwestern United States. C. immitis antigens which stimulate response of the T helper 1 (Th1) subset of T cells to infection are essential components of a vaccine against coccidioidomycosis. Recombinant glucanosyltransferase (rGel1) was expressed in E. coli and tested for its ability to protect C57BL/6 mice against a lethal intranasal (i.n.) challenge of the pathogen. A synthetic oligodeoxynucleotide which contained unmethylated CpG dinucleotides was shown to enhance a murine Th1 response and was used as an immunoadjuvant. Infected mice immunized subcutaneously with rGel1 were protected. We showed that 66.7% of the immunized mice survived more than 40 days after challenge compared to none of the control mice. Host cells recruited to sites of infection in the lungs of immunized and control mice were isolated by laser capture microdissection (LCM) and subjected to total RNA extraction and real time RT-PCR analysis of cytokine expression. Cytokine proteins secreted into bronchoalveolar lavage fluid were also examined by ELISA at 0, 1, 2, 3, 4, 7, and 14 days post-challenge. rGel1-immunized mice showed markedly elevated levels of IL-12 and IFN-γ compared to non-immune mice. Immunized mice also showed low levels of IL-5 and IL-10 mRNA/protein, while control mice revealed comparatively high levels of production of these two cytokines. We conclude that mice immunized with rGel1 develop a Th1-biased immune response to C. immitis infection, and that further evaluation of C. immitis rGel1 as a candidate vaccine against coccidioidomycosis is justified.

Mucosal subunit vaccines provide significant safety and efficacy advantages compared with traditional pathogen-based vaccines. However, lack of effective mucosal adjuvants approved for human use and cost-effective bioproduction systems currently limit their application. In order to develop cost-effective methods for nasally delivered vaccines, we have linked plant-based antigen bioproduction with development of an adjuvant/carrier that enhances mucosal immunity and antigen purification. Ricin B, the non-toxic galactose/galactosamine-binding subunit of ricin, was fused to a model antigen, green fluorescent protein (GFP), and expressed in tobacco plants and hairy root cultures to test for utility in mucosal vaccine delivery/adjuvancy. Intranasal immunization of mice with galactosamine-affinity purified ricin B-GFP recovered from tobacco root cultures triggered significant increases in GFP-specific serum IgGs. This strong humoral response was comparable to that observed following GFP immunization with cholera toxin adjuvant. GFP at the same concentrations but without an adjuvant was non-immunogenic. Induction of higher levels of IgG1 than IgG2a following ricin B-GFP immunization suggested the presence of a Th2 response. Serum and fecal anti-GFP IgA were also induced by immunization with ricin B-GFP. Our data suggest that ricin B can be used as an effective adjuvant and antigen carrier.
A phase I clinical trial was conducted in 12 patients that were positive for HPV-Type16 associated, anal intra-epithelial neoplasia (AIN). The therapeutic candidate, ZYCOS 1, is a microparticle-encapsulated plasmid DNA vaccine encoding for multiple HLA-A2 restricted epitopes. Each patient was treated with 4 intramuscular injections (dose / group: 50, 100, 200 or 400mg) of ZYCOS 1 at 3-week intervals. The trial reached completion and all data sets have been evaluated. The investigational agent was well tolerated in all patients. Histologic responses were observed in the upper dose levels (200 and 400mg). Additionally, the immunogenicity of this compound was screened. IFN-g ELISA (with in vitro stimulation) and ELISpot (with and without in vitro stimulation) assays were set up using patient PBMC samples from each timepoint. A significant proportion of the patients mounted an elevated immune response to the epitopes contained within the formulation. These data support further studies to evaluate the therapeutic applications of the vaccine candidate.

**Results:**
- **Conclusions:** Prime-boost vaccination with ALVAC-HIV (vCP1521) priming with AIDSVAX B/E boosting in Thailand: Safety and Immunogenicity Results

**Methods:** The study was a double-blind, randomized, placebo-controlled trial. Volunteers were enrolled and divided into two groups based on the dose of AIDSVAX B/E.

**Background:** A strategy to stimulate HIV-specific cellular and humoral antibodies uses vector vaccine priming and subunit boosting. vCP1521 is a recombinant canarypox vector expressing subtype E HIV-1 gp120 (92TH023) linked to transmembrane anchoring portion of gp41 (strain LAI), HIV-1 gag and protease (LAI strain). AIDSVAX B/E is a bivalent HIV gp120 envelope glycoprotein. The study was a double-blind, randomized, placebo-controlled trial. Volunteers were enrolled and divided into two groups based on the dose of AIDSVAX B/E.

**Results:** Most reactogenicity was mild to moderate. No serious adverse events were attributable to vaccine. Among recipients of the 600 mg boost, 71% and 98% of vaccine recipient volunteers had neutralizing antibody to subtype E and B TCLA HIV strains, 71% had lymphocyte proliferation to gp120 CM24, while 49% had proliferation to gp120 MN.

**Conclusions:** Prime-boost vaccination with ALVAC-HIV and AIDSVAX B/E appears safe, well-tolerated and able to induce both humoral and cellular HIV-specific immune responses. These results support the planning for the next phase III HIV vaccine trial in Thailand.
**Characterization of the Efficacy and the Immune Response of Nabi® StaphVAX™ (A Bivalent Staphylococcus aureus (S. aureus) Type 5 and Type 8 Capsular Polysaccharide (CP) Conjugate Vaccine) in Hemodialysis Patients**

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1Research, Nabi, Rockville, MD, 2Research, Nabi, Rockville, MD, 3Nabi, Rockville, MD

**Introduction:** Nabi® StaphVAX® results from a Phase III efficacy trial in hemodialysis patients showed that the vaccine significantly reduced S. aureus bacteremias through 10 months post-vaccination. Efficacy decreased, parallelizing waning population geometric mean types 5 and 8 antibody levels.

**Methods:** We evaluated the quality and functionality of the S. aureus capsular antibodies before and after the loss of the efficacy of the vaccine. The affinity and functionality of antibodies from serum samples of patients at 6 weeks, 6 and 12 months after vaccination were evaluated utilizing the thioycanate dissociation method and an in vitro opsonophagocytosis assay utilizing a HL-60 cell line, respectively.

**Results:** There appears to be no change in antibody affinity throughout the 12-month period. To assess opsonophagocytosis, vaccine induced antibodies from hemodialysis were compared to those from healthy adults. An excellent, linear correlation existed (r > 0.98) at all time points. Further, the specific opsonic activity of antibodies from hemodialysis patients was similar to that observed with antibodies from healthy adult vaccinees.

**Conclusions:** These data suggest that protective levels of antibodies are host-related and that loss of efficacy despite relatively high levels may be related to impaired neutrophil function. Because of a better immunocompetency in other at-risk for S. aureus infection populations, such as surgery patients, StaphVAX® is expected to demonstrate superior efficacy compared to that obtained in hemodialysis patients.

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**QS-21 Enhances the Response to Pneumococcal Conjugate Vaccine in the Elderly**

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Polysaccharide vaccine is effective in the elderly but immune responses in these individuals are suboptimal. In a previous study, we were not able to demonstrate any advantage of pneumococcal conjugate vaccine over polysaccharide vaccine in elderly adults. Since the advent of QS-21 has been shown to increase the antibody response to a GM2-KLH glycoconjugate melanoma vaccine, we performed a pilot study evaluating addition of QS-21 to a pneumococcal conjugate vaccine. Thirty previously unvaccinated adults over age 65 were randomly assigned (10 subjects per group) to receive a single IM dose of 2.5-valent pneumococcal polysaccharide vaccine (Pnu-Immune 23), or to two doses separated by 120 days of either 7-valent CRM197-conjugated vaccine (Prevnar, serotypes 4, 6B, 9V, 14, 18C, 19F, 23F), or Prevnar plus 50 µg of QS-21. Serum antibody responses to the 7 serotypes were assessed on days 0, 30, 120, and 150. All vaccines were well tolerated, and addition of QS-21 to Prevnar did not result in markedly increased local pain. Subjects receiving QS-21 had significant serum antibody responses to more serotypes (median 6 out of 7 possible responses) than did those receiving Prevnar alone (median 2.5 serotypes, P=0.01) or Pnu-Immune (median 2 serotypes, P=0.07). In addition, subjects receiving QS-21 had higher post-vaccination titers for most serotypes, and a greater response to revaccination than did those receiving Prevnar alone. These results indicate that QS-21 is well tolerated and could be useful when combined with polysaccharide conjugate vaccines in the elderly.

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**Safety and Immunogenicity of 26-valent Group A Streptococcus (GrAS) Vaccine in Healthy Adults**


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We performed a phase I trial of a 26-valent GrAS vaccine in 30 healthy adults. This vaccine comprises four recombinant proteins adsorbed to alum. Together, these contain tandem N-terminal peptides from M proteins of 26 common pharyngitits, invasive, and/or rheumatogenic serotypes. Subjects were screened for good general health and underwent baseline cardiac auscultation, echocardiography, ECG, and screening for human tissue-cross-reacting antibodies. Intramuscular vaccine (400 µg) was administered at 0, 1 and 4 months, with clinical and laboratory follow-up for safety and assay of type-specific M antibodies by ELISA and opsonization tests. Most adverse events were at the injection site. The incidence of erythema, induration, tenderness and pain was similar to other alum-adsorbed vaccines in adults. There has been no clinical or laboratory evidence of rheumatogenicity or nephritogenicity, and no induction of human tissue-reactive antibodies. Type-specific M antibody responses against a mean of 23.4 (median 24.5) of the 26 vaccine serotypes were apparent after the second dose and boosted strongly after the third. This represents coverage of serotypes responsible for a weighted mean of 84.5% (median 86.1%) of pharyngitis episodes in a 2000-2001 North American survey. Results of antibody mediated opsonization assays using vaccine and potential cross-reacting serotypes will be discussed. In this trial, a 26-valent GrAS vaccine was safe and immunogenic in healthy adults; phase II trials are warranted.

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**Two Paediatric Doses of Twinrix or Recombivax Provides Nearly as Good Protection as Three Doses to Pre-teensagers**

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**Background:** Nearly all pre-teensagers are protected after three paediatric doses of hepatitis B vaccine and very high GMT are observed. This raises the possibility that two paediatric doses be sufficient to protect them. This study measured the immunogenicity of two paediatric doses of Twinrix Paediatric and Recombivax HB 2.5 µg administered 6 months apart to 9 year-old children.

**Methods:** 357 children received Recombivax-HB 2.5 µg and 347 Twinrix paediatric (360 ELU HAV antigen; 10 µg HbsAg) at months 0 and 6. Immunogenicity was measured by ELISA one month after the second dose. Results were compared with those obtained with 3 doses schedules of Recombivax 2.5 µg and Engerix 10 µg in historical controls in the same population.

**Results:** The seroprotection rate (≥ 10 mUI/ml) against Hepatitis B was 94.4% (CI 91.5-96.3) for Recombivax-HB and 96.5% (CI 91.4-98.0) for Twinrix. The GMTs were respectively 742 mUI/ml (CI 593-929) and 3248 mUI/ml (CI 2579-4091). This compared with the following results with three doses in historical controls: a seroprotection rate of 99.2% (CI 98.3-99.6) and a GMT of 3393 mUI/ml (CI 3072-3747) with Recombivax-HB, a seroprotection rate of 98.9% (CI 98.2-99.4) and a GMT of 7307 (CI 6582-8110) with Engerix-B.

**Conclusions:** It is possible to use only two paediatric doses of these hepatitis B vaccines in pre-teensagers with results nearly as high as with the three doses schedule.
Building Vaccines from Immunogenic Consensus Sequences

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Background: Broad T cell responses to dominant and subdominant epitopes are associated with better control of HIV-1 infection. However, HIV-1 epitope variability impedes the development of a global HIV vaccine.

Methods: 1000 short, highly conserved sequences were selected from more than 55,000 HIV-1 sequences, then scored against all 74 unique EpiMatrix Class II matrices, and ranked by (1) number of HIV-1 strains represented; (2) EpiMatrix score; and (3) promiscuity (number of unique MHC motifs contained in the peptide). Candidate 20 to 23-mer epitopes were then designed by overlapping the highest ranked peptides and sequentially extending each flank with a conserved and potentially immunogenic 9 mer. The resulting “immunogenic consensus” sequence was entirely novel, even though each overlapping 9-mer contained in the sequence was highly conserved in our HIV-1 variant database.

Results and Conclusion: Eight of the top 100 immunogenic consensus peptides selected using this approach overlapped with published T helper epitopes. Three of the 15 highest ranked “immunogenic consensus” epitopes were confirmed in ELISpot assays using PBMC from healthy HIV-1 infected individuals (effector frequency 1:2,000 to 1:4,000 PBMC).

These epitopes, ranked 2, 3 and 14, were conserved in 2218, 893, and 1231 HIV-1 env sequences, respectively. All are novel, one contains a defined MHC A3-restricted CTL epitope. 60% of the remaining 100 epitopes are expected to be immunogenic.

Profilig Gene Expression Changes Associated with Eimeria Infections Using High-throughput cDNA Microarrays

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Global transcriptional changes induced in the chicken intestinal intraepithelial lymphocytes (IELs) in response to an intracellular parasitic infection were investigated using nylon and glass cDNA microarrays. Chicken EST cDNAs were selected from the cDNA libraries developed from the activated T-lymphocytes and macrophages (www.chicken.udesl.edu). To identify host genes induced by Eimeria infections, we hybridized cDNA microarrays with intestinal mRNA samples obtained from chickens infected with E. acervulina or E. maxima. Among the 2,600 EST clones screened, 419 clones were selected on the basis of at least 1.5 folds increased or decreased expression over the uninfected controls. Following infection with E. acervulina or E. maxima, 9.5% and 2.3% of total EST clones tested were increased, and 10.7% and 2.3% were repressed following primary and secondary infections, respectively. In general, E. acervulina infection resulted in a greater change in local gene expression than E. maxima infection. The detailed analysis of local changes in host gene expression following Eimeria infections will enhance our understanding of host-parasite immunobiology leading to protective immunity.

The Role of HLA Genes in Measles Vaccine Virus (MVV) Antibody (Ab) Hyper-Response


Mayo Vaccine Research Group, Mayo Clinic and Foundation, Rochester, MN

Limited information on the influence of HLA genes on Ab hyper-response to MVV is available. We studied the association between Ab hyper-response to MVV and HLA alleles among US schoolchildren between the ages of 5 and 12 years, who received one dose of MMR-II vaccine (Merck). We performed HLA typing of genomic DNA in 93 MVV normal responders (NR) and 77 hyper-responders (HR, upper 10th percentile of Ab response). This information increases our understanding of MVV-induced immune response based on genetic polymorphism of the HLA genes.

Differential HLA Gene Expression in Responders and Non-Responders to Measles Vaccine Virus (MVV) using GeneChip Expression Arrays

N. Dhiman, R. B. Guerrero, R. M. Jacobson, D. J. O’Kane, G. A. Poland

Mayo Vaccine Research Group, Mayo Clinic and Foundation, Rochester, MN

The genetic determinants involved in variable immune responses to MVV are poorly understood. We conducted a study using Human U-95A GeneChip expression arrays (Affymetrix) in five healthy humans immunized with a “booster” dose of MVV (Merck) to determine if serologically distinct subjects exhibit differential expression of HLA genes. Subjects were classified as non-responders [n=2] and responders [n=3] based on MVV-specific serum IgG levels. Following immunization, total RNA was isolated from PBMCs at day 0, 7 and 14 using the Qiagen RNeasy Midi kit. The HLA related targets on Chip A were analysed using Microarray Analysis Suite 5.0 and Spotfire software. Out of a total of 38 HLA genes, 6 genes exhibited differential expression on day 7 and 14 post vaccination as compared to day 0 in the two groups studied. We observed an increased expression of the HLA Class I-B (p-value =0.0002), HLA Class II cluster of DMA, DMB, HLA-Z1, IPP2, LMP2, LMP7, TAP1, TAP2 (p-value =0.0007), HLA-DR (p-value <0.0001) and HLA-DP (p-value <0.0001) and decreased expression of HLA class I MICB molecule (p-value =0.001) and MHC class III HSP 70 (p-value = 0.01) on day 7 or 14 post vaccination in responders as compared to the non-responders. These results suggest a possible association between antibody response and differential HLA gene activation and may explain one of the potential mechanisms underlying MVV non-response.
on Vaccine Research

Abstracts of Submitted Presentations

S33 Cytokine mRNA Expression Profile after Measles Vaccine Virus Immunization Failure
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Background: Development of protective immune response depends on many factors. To understand cytokine gene regulation after measles vaccine virus (MVV) immunization, we compared cytokine mRNA expression in two populations.

Methods: We recruited 4 subjects categorized as normal antibody (Ab) responders (N, n=2), and low Ab responders (LR, n=2) based on serum measles-specific IgG antibody level. Subjects underwent phlebotomy; peripheral blood mononuclear cells (PBMCs) were isolated and cultured in the presence or absence of 2000 TCID50 of MVV. Total RNA was extracted (Qiagen RNeasy extraction kit) after 6 hours, 3 and 4 days of incubation. The mRNA expression levels for 12 cytokines (IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p35, IL-12p40, IFN-g, and TNF-a) were assayed by TaqMan RT-PCR assay.

Results: Normal responders have a higher overall cytokine mRNA level (P <0.0001) and a larger fold change increase (12 vs. 1.5 Fold) to unstimulated and stimulated PBMCs than low responders (P <0.007). Simultaneous response of Th1 and Th2 cytokines in all subjects was found.

Conclusions: Despite that normal and low Ab responder’s cytokine mRNA expression levels correlate with their Ab production phenotype, an absence of predominance of either Th1 or Th2 cytokine mRNA differentiation after MVV stimulation exists, suggesting that at some later point in the T cell differentiation after MVV stimulation exists, suggesting that at some later point in the T cell differentiation, blockage occurs, which underlies all variations immune response phenotypes after measles vaccination, including vaccine failure.

S34 Improved Biological Activities for Novel HIV-1 Envelope Vaccine Candidates Generated by DNA Shuffling
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Background: DNA shuffling captures the power of genetic recombination in a simple test tube format. This technology is an effective approach for generating high quality libraries of chimeric sequences that encode proteins with novel and improved biological properties. We have used DNA shuffling to create chimeric HIV-1 envelope proteins and have screened them in various protocols for evaluation as vaccine candidates.

Methods: Envelope genes encoding gp160, gp120, and a gp120core construct from HIV-1 primary isolates were used in shuffling reactions to generate recombinant libraries. Protein expression from the shuffled clones was verified using immunostaining of transfected cells or immunoblotting of culture supernatants. Secretion of parental envelope proteins was assessed using monoclonal antibodies and flow cytometry. The ability of secreted gp120 proteins to bind a monoclonal neutralizing antibody was evaluated by immunoblotting. The immunogenicity of envelope-expressing DNA clones was evaluated in mice using a DNA vaccine format.

Results: Sequence analyses of the shuffled clones in libraries indicated that all clones were highly chimeric and contained fragments from the parental sequences. A number of the shuffled clones showed improved immunogenicity compared to the parental clones. Some of the shuffled gp120 clones bound more neutralizing antibody than the parental clones.

Conclusion: Novel HIV-1 envelope-coding genes generated by DNA shuffling produced proteins with enhanced biological activities. Further evaluation of these novel antigens for induction of cross-reactive neutralizing activities against primary HIV strains, the absence of which poses a major barrier to HIV vaccine development, is in progress.

S35 New Uses for Licensed Vaccines: Evaluation of the Japanese Encephalitis Virus Vaccine and the NY99 West Nile Virus Genomes for Cross-reactive T Cell Epitopes
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Background: Given the recent expansion of WNV in the United States, consideration may be given to using the licensed JEV-NN vaccine to protect at-risk individuals against WNV. Conservation of T cell epitopes between the genomes of the Japanese encephalitis virus vaccine (JEV-NN) and West Nile Virus (WNV) might explain some aspects of the cross-protective effect of JEV-NN immunization observed in animal models.

Methods: We evaluated JEV-NN and WNV (strain NY99) for putative CTL epitopes reported here would stimulate the putative HLA A2-, HLA A3-, HLA A11-restricted JEV/WNV epitopes reported here would stimulate the putative HLA A2-, HLA A3-, HLA A11-restricted by more than one HLA molecule. Based on this analysis, we published CTL epitopes. Many of these cross-reactive CTL epitopes were identified using a number of bioinformatics (SignalP, Prosite, and EpiMatrix).

Results: 29 peptides of the highest number of MHC binding motif matches. T cell responses to selected peptides were evaluated in gamma-interferon release ELIspot assays and in T cell proliferation assays, using peripheral blood monocytes (PBMC) obtained from healthy, Mtb-infected (Mtb immune) donors.

Results and Conclusion: The number of potential peptides to be screened was reduced to 97 candidates from more than 1.3 million by bioinformatics (SignalP, Prosite, and EpiMatrix). 29 peptides of the highest ranking candidates (by number of MHC binding motifs) in this list of 97 were selected for analysis. Twelve (65%) of these 17 peptides that could be synthesized and studied in vitro as T cell responses to selected peptides in vitro. An extended analysis, using all class I and II motifs available in the EpiMatrix repertoire, can also be performed.

Conclusion: This type of analysis may be of use to groups formulating recommendations regarding the use of the licensed JEV-NN vaccine to protect high-risk subjects during an epidemic of WNV. This approach may be used to evaluate and expand the use of other licensed vaccines.

S36 Analyzing Mycobacterium Tuberculosis Proteomes for Candidate TB Vaccine Epitopes
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Background: We used web-based and proprietary bioinformatics tools to analyze proteomes selected from the genomes of Mycobacterium tuberculosis (Mtb) 1551 and H37Rv for T cell epitopes.

Methods: EpiMatrix, a computer-driven pattern-matching algorithm, was used to analyze the sequences of putative secreted proteins derived from the Mtb 1551 and H37Rv genomes for regions that contained a high number of MHC binding motif matches. T cell responses to selected peptides were evaluated in gamma-interferon release ELIspot assays and in T cell proliferation assays, using peripheral blood monocytes (PBMC) obtained from healthy, Mtb-infected (Mtb immune) donors.

Results: 94% highly conserved peptides were identified. 300 of these scored in the 99th percentile of EpiMatrix scores, as high or higher than published CTL epitopes. Many of these cross-reactive CTL epitopes were identified using a number of bioinformatics (SignalP, Prosite, and EpiMatrix). 29 peptides of the highest ranking candidates (by number of MHC binding motifs) in this list of 97 were selected for analysis. Twelve (65%) of these 17 peptides that could be synthesized and studied in vitro as T cell responses to selected peptides in vitro. An extended analysis, using all class I and II motifs available in the EpiMatrix repertoire, can also be performed.

Conclusion: This type of analysis may be of use to groups formulating recommendations regarding the use of the licensed JEV-NN vaccine to protect high-risk subjects during an epidemic of WNV. This approach may be used to evaluate and expand the use of other licensed vaccines.
Addition of CpG-containing Oligonucleotides to a Hepatitis B Vaccine Markedly Enhances the Antibody Avidity Maturation Process in Healthy Adults

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Objective: A 24-mer oligonucleotide containing 3 CpG motifs (CpG 7909) significantly enhanced anti-HBsAg responses in adult volunteers vaccinated at 0, 4, and 24 weeks with 20μg of alum-adsorbed HBSAg (Engerix-B®, Glaxo SmithKline) and added CpG (500μg) compared to controls. We asked whether CpG 7909 stimulated the production of anti-HBsAg Ab of lower, similar, or higher functional capacity.

Methods and Results: The avidity of HBSAg-specific Ab was determined by ELISA elution assay using thiocyanate (NH4SCN) as a chaotrophic agent. In controls, mean IgG1 avidity index (AI) reached a plateau (1.2M) after the 3rd dose. In the CpG group, it increased from 0.46M (wk 8) to 0.95M (wk 24), 1.7M (wk 26) and 1.8M (wk 52), indicating a significantly enhanced (p=0.001 at wk 52) avidity maturation process. This was confirmed by the proportion of high avidity (eluted > 2M NH4SCN)anti-HBsAg IgG1 Ab, which increased from 8% to 40% at wk 52 in the CpG group, compared to only 18% in the control group (p=0.012). Avidity of anti-HBsAg IgG3 Ab, present at lower levels, was similar in both groups.

Conclusions: Addition of CpG 7909 to Engerix-B® enhanced the avidity maturation process which results from B cell proliferation, germinal center differentiation, hypermutation and clonal competition.

No Novel Adjuvant Formulations Containing a Thermal-setting Block Copolymer and Selected Immunomodulators Elicit Potent and Long Lasting Antibody Responses in Mice

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We have been investigating novel adjuvant formulations for use as vaccine delivery vehicles. These formulations consist of the block-copolymer, Pluronic® F127, which exhibits reverse thermo-gelation characteristics, in combination with selected immunomodulators. The pluronic potentially stabilizes antigenic conformation within the matrix and provides a depot for antigen release at body temperature. In these studies, we have evaluated protein antigens formulated with F127 in combination with CpG motifs or chitosan, as examples of immunomodulators, and we have compared these formulations to more traditional adjuvants.

Tetanus toxoid (TT) and recombinant anthrax protective antigen (rPA) were used as antigens. Mice were immunized once subcutaneously with various formulations and antibody responses were measured by ELISA. Antibody titers to both rPA/F127/chitosan and rPA/F127/CpG were significantly higher than those elicited by rPA/alum (p < 0.05 at 8 weeks, by Mann-Whitney Rank Sum Test) although rPA/F127/chitosan and rPA/F127/CpG responses did not significantly differ from each other (p=0.05). In addition, the response to rPA/F127/CpG was significantly higher than that to rPA/CpG alone (p = 0.026 at 4 weeks). In animals immunized with TT/F127/CpG, the IgG1 antibody response was significantly enhanced compared to that of mice immunized with TT/CpG alone (p=0.029 at 8 weeks) and the response was also significantly higher than that elicited by TT/CpG/IFA (p=0.029). These studies suggest that a block-copolymer approach could have utility in delivering a variety of clinically useful vaccines. Further studies are underway targeting an improved anthrax vaccine.
Background: Nonprogression of disease in HIV-infected individuals correlates with strong, durable HIV-specific cytotoxic T lymphocyte (CTL) responses. We have used recombinant SV40 vectors (rSV40) to elicit strong murine cytotoxic and humoral immune responses against HIV-1 gp120. Cytokines like IL-15 promote CTL memory, and so may accelerate and augment cytolytic responses. We investigated whether delivering IL-15 in a rSV40 augmented anti-gp120 immune responses.

Methods: Mice received SV(gp120) +/- SV(mIL-15) monthly. Cloned gp120-expressing P815 cells were used as targets in a cell-based ELISA (CELISA) to measure antibodies binding to gp120. The same cells were used in a 51Cr-release assay to determine gp120-specific cytolytic responses.

Results: A single injection, or combination of injections generally elicited antibody responses. However, administering IL-15 before SV(gp120) significantly affected cytotoxic responses: mice given IL-15 3d before gp120 made very high responses at low effector:target ratios (20:1, 10:1) (> 50% specific lysis). Inoculating SV(mIL-15) simultaneously with, or following, SV(gp120) did not augment cytolytic responses (< 20% specific lysis).

Conclusions: As few as 2 immunizations with SV(gp120) elicited both humoral and cell-mediated immune responses against HIV-1 gp120. Administering SV(mIL-15) 3d before SV(gp120) in this regimen succeeded in promoting CTL memory. rSV40 vectors delivering target antigens and immunostimulatory cytokines may be useful in devising vaccines against HIV-1.

Background: During the 2000-2001 influenza immunization campaign, a newly identified oculo-respiratory syndrome (ORS) was detected in Canada. Vaccine-related investigations found unexpected micro-aggregates of unsplit virosomes in the implicated manufacturer's product. We report the recurrence risk of ORS subsequent to changes in the virus splitting process for 2001-2002.

Methods: Double-blind randomized placebo controlled cross-over trial of up to 150 persons vaccines both in patients with and without a prior episode of ORS. This comparison Fluviral to Vaxigrip was 1.3 (CI 95% 0.8-2.1). Patients with prior episode of ORS had an aOR of 10.0 (CI 95% 5.2-19.1) when compared to those without history.

Conclusions: One quarter of persons who had previously experienced ORS experienced recurrence despite changes to the virus splitting process. These findings have implications for the re-immunization of affected persons. The immune mechanism for ORS remains uncertain.

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Conclusions: One quarter of persons who had previously experienced ORS experienced recurrence despite changes to the virus splitting process. These findings have implications for the re-immunization of affected persons. The immune mechanism for ORS remains uncertain.
S45 Oral Immunogenicity of Human Papillomavirus Virus-like Particles Expressed in Potato
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Background: Human papillomavirus (HPV) virus-like particles (VLPs) are promising vaccine candidates for controlling anogenital HPV disease, and are now being evaluated as a parenteral vaccine for immunoprophylactic efficacy in humans. We previously reported that purified VLPs are also immunogenic when administered orally in combination with E. coli LT(R192G). Here we examine oral immunogenicity of VLPs expressed in transgenic potato (Solanum tuberosum, cv Desiree).

Methods: HPV-11 L1 coding sequences were introduced into the potato genome by Agrobacterium tumefaciens-mediated transformation. Mice (N=5-10/group) received four meals (5grams) at weekly intervals of non-transgenic potato, or transgenic potato with or without LT(R192G) (5ug). Some animals were then boosted orally with a sub-immunogenic dose of purified VLPs (0.5ug) with LT(R192G) (5ug). Sera were collected at various timepoints and evaluated by ELISA.

Results: Mice fed transgenic potato with LT(R192G) and then boosted orally with purified VLPs with LT(R192G) developed anti-VLP serum antibody titers that were significantly higher than titers observed in animals fed either non-transgenic or transgenic potato without adjuvant, with or without oral boosting (P<0.05).

Conclusions: HPV-11 transgenic L1 potato co-administered with LT(R192G) was immunogenic in mice and exerted a strong priming effect for subsequent oral booster immunization with an otherwise sub-immunogenic dose of purified VLPs. This constitutes the first report of an HPV VLP vaccine produced in transgenic edible plants that induces a potentially protective response in animals.

S46 A New Subunit Vaccine for Tuberculosis Containing the Dissemination Factor, HBHA
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The mycobacterial Heparin-Binding Hemagglutinin (HBHA) is a surface-exposed protein involved in dissemination of Mycobacterium tuberculosis (Mtb) from the lungs. Native Mtb-expressed HBHA (nHBHA), purified by heparin affinity chromatography elicits immune responses in Mtb-infected patients. Evidence suggests that Mtb posttranslationally modifies the lysine-rich repetitive domain of HBHA, which mediates adherence of mycobacteria to epithelial cells via the recognition of sulfated glycoconjugates. To investigate the potential use of HBHA as a TB vaccine, mice were vaccinated 3 times subcutaneously with 5 mg each of nHBHA or purified (unmodified) recombinant HBHA (rHBHA) adjuvanted with dimethyltetradecylammonium bromide (DDA) and monophosphoryl lipid A (MPL), and challenged 4 weeks later with aerosolized Mtb. Mice vaccinated with nHBHA exhibited a significant reduction (p<0.01) of CFUs in lung and spleen (-0.83 and -0.69 log, respectively) at 28 days post-challenge compared with adjuvant-vaccinated controls. Mice immunized with rHBHA displayed a reduction (p<0.05) of CFUs only in the spleen (-0.56 log) compared with controls and exhibited increased lung pathology compared with both BCG and nHBHA immunized mice. A vigorous serum IgG response against both forms of HBHA was observed in mice receiving the nHBHA, whereas mice receiving rHBHA responded only modestly to HBHA. Mice vaccinated with nHBHA using the same strategy and challenged intravenously with 10^3 Mtb, were protected to a similar extent compared with BCG-vaccinated mice. Taken together, these results indicate that nHBHA is a promising new candidate antigen as a component in a subunit vaccine for TB.

S47 Completely Stable Liquid Vaccines
B. J. Rosser, S. D. Sen
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Background: Current vaccines need refrigeration. However, twelve vaccines have been stabilised by drying in a sugar glass but these then require rehydration. What is needed is a completely stable liquid vaccine that requires no refrigeration and that can be injected without any preparation.

Methods: Tetanus vaccine was spray dried as sugar-glass microspheres; the powder was then suspended in an anhydrous, injectable, liquid perfluorocarbon (PFC). PFCs have advantages over oils. They do not oxidise or become rancid, are chemically non-reactive, and have high density, low viscosity and low surface tension that makes them ideal to inject either via a needle or a jet injector. Their extreme lack of toxicity also modifies the lysine-rich repetitive domain of HBHA, which mediates dissemination of Mtb from the lungs. Native Mtb-expressed HBHA (nHBHA), surface-exposed protein involved in dissemination Factor, HBHA

Conclusions: Most if not all vaccines are suitable for sugar-glass stabilisation and then formulating as stable suspensions in PFCs, which can be stored at any ambient temperature. They can be pre-packaged on the shelf in ready-to-inject single dose devices.

S48 Immunogenicity and Efficacy of a Novel Shigella Invasin Complex /ETEC Colonization Factor Bivalent Vaccine in Mice
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Two common causes of diarrhea for travelers and troops stationed in foreign countries are Shigella and enterotoxigenic Escherichia coli (ETEC). Subunit vaccines for Shigella and ETEC include the Shigella invasin complex (Invaplex), consisting of Ipa proteins and LPS, and for ETEC, colonization factors (CF) and potentially heat labile toxin. We have combined the S. flexneri 2a Invaplex vaccine with ETEC CF for the development of a bivalent Shigella/ETEC vaccine. This combination uses the innate adjuvanticity of the Invaplex to promote the immune response to the co-delivered ETEC antigens. Mice were immunized intranasally three times at two-week intervals with S.flexneri Invaplex 24 (5ug) mixed with various amounts of ETEC CS6 (0 ug, 0.1 ug, 1.0 ug and 5.0 ug). The combination of Invaplex with CS6 produced higher levels of IgA and IgG serum antibodies in mice than CS6 alone. Antibody levels to Shigella LPS and Ipa proteins were similar in animals immunized with Invaplex plus CS6 or Invaplex alone. Mice immunized with Invaplex and CFA/I also produced high IgA and IgG levels to both Shigella antigens and ETEC CFA/I. Using the mouse lethal lung model for Shigella, mice immunized with the bivalent Shigella/ETEC vaccine were protected from a lethal Shigella challenge at levels comparable to mice receiving only the Invaplex vaccine. The combination of the Shigella Invaplex vaccine with ETEC CF may lead to a broadly protective vaccine against two highly prevalent diarrheal agents.
Efficacy of new-type LPS Shigella sonnei vaccine SHIGELLVAC was assessed in double-blind randomized placebo-controlled clinical field trials. Under the guidance of Russia’s NCA inhabitants living in two settlements of mid-Volga region with annual high incidence rate of Sh. sonnei infection in the summer-autumn season were immunized. The target population was composed of subjects between 3 and 60 years old. Volunteers were randomly allocated to two groups: 1802 received one injection of Sh. sonnei vaccine; 1266 received one injection of placebo. Only minimal side-reactions (local redness, painfullness) were registered after subcutaneous immunization of volunteers with 50 mg of vaccine. We defined Sh. sonnei shigellosis based on clinical symptoms (diarrhoea) associated with positive faecal culture for Sh. sonnei. Each group of volunteers was followed up 6 months from July 2001 to January 2002. Cases of culture-proven Sh.sonnei shigellosis occurred in both groups. We found that the protective efficacy of Sh. sonnei LPS vaccine was 92.4% during 6 month period of disease surveillance. Immunization with new type vaccine confers protection of civic population during season with maximal risk of spreading shigellosis Sonnei infection.
P1 Broad Spectrum Pulsed Light-Inactivated Herpesvirus Antigen Preparations for Serological Assays and Potential Virus Vaccines

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The Viral Immunology Center focuses on research and diagnosis of B virus, a zoonotic herpesvirus which naturally infects macaques and causes a fatal infection in humans. Thus, the safety and efficacy of antigen preparation is of chief concern. The PureBright® Anti-Pathogen Research (APR) System was used to inactivate lysates of herpesvirus-infected cells for antigen production. The APR system utilizes Broad-Spectrum Pulsed Light (BSPL) generated from lamps containing visible, ultraviolet and infrared wavelengths in ratios similar to solar radiation unfiltered by the Earth's atmosphere. We used herpes simplex virus type 1 (HSV-1) and herpesvirus papio 2 (HVP-2) as models to assess the efficacy of BSPL inactivation of human and simian herpesviruses. The effect of BSPL on virus inactivation was tested by viral plaque assay in cell culture. The effect of BSPL on antigenicity was tested by antibody and antigen capture ELISAs. We achieved a sterility assurance level of 10^-6 PFU/ml with a total energy of 3.7 J/cm2 and 4.2 J/cm2 (for HSV-1 and HVP-2, respectively). At these energies, greater than 70% of the antigenicity was retained. Further, there was no apparent degradation of BSPL-inactivated antigen preparations as demonstrated by SDS/PAGE analysis. We therefore conclude that the BSPL-inactivated antigen can replace the detergent-solubilized herpesvirus antigen preparations currently used in our laboratory. Based on the preservation of antigenicity in the BSPL-treated virus preparations, we believe that they will serve as good immunogens and candidates for future vaccine studies.

P2 Liposomal gd Vaccine (LipgD-HD) Protects BALB/c and C57BL/6 Male Mice Challenged Intrarectally (Irec) with Herpes Simplex Virus (HSV2)

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Prior studies showed LipgD-HD protected female mice challenged intravaginally with HSV2. To study LipgD-HD efficacy in male mice, an Irec male HSV2 infection model was developed. Wks 1, 4, and 8 mice were vaccinated subcutaneously with buffer, 6 or 8mg/kg of gd(1-23aa) protein fused with a hydrophobic domain in phospholipid/cholesterol liposomes <200nm. Wk 9, 7 mice/group were Irec challenged with 1x10^7pfu HSV2 and monitored 90d for morbidity. Sera and splenic T cells were collected from 5 mice/group to determine neutralizing antibody (NA) titer, T cell proliferation and cytokine production after 6d incubation with gd(1-23aa). By d10-13, all control mice died with severe intrarectal and neurological signs. Clinical signs were minimal in vaccinated mice with 57% (6mg/kg) and 43% (8mg/kg) survival for BALB/c mice and 57% survival for C57BL/6 mice at both doses. Compared to controls, NA titers were 15X (BALB/c) and 20X (C57BL/6) higher, and T cell proliferation was 2.7X (BALB/c) and 2.6X (C57BL/6) higher in 6mg/kg vaccinated mice. IL-4, IL-2 and gamma interferon levels in 6mg/kg vaccinated mice were 3.7X, 2.7X, 5.8X higher for BALB/c, respectively and 2.8X, 2.5X, 5.1X, higher, respectively, for C57BL/6. In conclusion, LipgD-HD stimulated B and T cell responses that minimized Irec HSV2 infection in BALB/c and C57BL/6 male mice.

P3 Enhanced Antibody Response and Protective Capacity of Japanese Encephalitis Virus DNA Vaccine With a Combination of Plasmids Expressing Envelope and Nonstructural Protein Genes

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Antibody response has been implicated as the critical effector in protective immunity against Japanese encephalitis virus (JEV). A DNA vaccine (pE) expressing the envelope (E) protein of JEV has been demonstrated that protects mice against a lethal challenge by inducing anti-E neutralizing antibodies. The immune response induced by nonstructural protein (NS) 1 also provides protection against JEV challenge. To determine the protective capacity of multigenic DNA vaccine, we used three plasmids encoding NS1-2A (pNS1-2A), NS3 (pNS3), and NS5 (pNS5) gene combined with pE to immunize mice by gene gun. Combination of E and NS gene plasmids provides better protection than them induced alone. We also found codeelivery of pE and nonstructural gene plasmids can enhance anti-E antibody titers, interferon-gamma production, and cytokotoxic T cell response. Analysis of anti-E antibody isotype, a significant increase of IgG2a was observed in mice coimmunized with pE and nonstructural gene plasmids. Transfer with antisera but not spleen cells of immunized mice conferred the protection induced by pE plus pNS1-2A. By providing the evidence that immune response and protection were enhanced by a combination of E and NS gene plasmids, these studies give the basis to design the multigenic JEV DNA vaccine.

P4 Enhanced Antibody Response and Protective Capacity of Japanese Encephalitis Virus DNA Vaccine With a Combination of Plasmids Expressing Envelope and Nonstructural Protein Genes

C. Pan1, H. Chen2, H. Huang3, H. Yen3, M. Tao3
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OM-174 in vivo may involve activation via more than one TLR. This suggests that it may also signal via human TLR-2. The potent responses to OM-174 of NF-kB in transfected HEK-293 cells, of BM-DC to OM-174, although other signalling systems may also play a role. Upregulation by OM-174 of NF-kB in transfected HEK-293 cells, suggests that it may also signal via human TLR-2. The potent responses to OM-174 in vivo may involve activation via more than one TLR.

**P6** Immunostimulatory Actions of SGN-00101 and the Separation of These Effects from Lipopolysaccharide

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Immunization with SGN-00101, an Hsp fusion protein comprised of Mycobacterium bovis BCG Hsp65 linked to HPV16 E7, results in eradication of HPV E7 expressing cells in both preclinical models and human phase II clinical trials. Effective treatment with SGN-00101 requires activation of CD8+ T cells. Since SGN-00101 is active without adjuvant, we wished to determine if HspE7 could stimulate the release of proinflammatory cytokines from cells of the innate immune system.

THP-1 cells were stimulated with SGN-00101 or LPS, SGN-00101 or it’s component parts. For desensitization studies, cells were stimulated with LPS or SGN-00101, washed and then restimulated. For serum dependence studies, THP-1 cells were stimulated with SGN-00101 or LPS in 10% or 1% FCS. TNFα production was assessed by ELISA. HspE7, but not Hsp65, E7 (or the admixture) induced the release of TNFα from THP-1 cells. While SGN-00101 was able to elicit TNFα release from THP-1 cells cultured in low serum containing media, LPS did not elicit TNFα under these conditions. Pretreatment with SGN-00101 desensitized the THP-1 cells to restimulation with either LPS or SGN-00101, while LPS pretreatment resulted in complete desensitization to restimulation with LPS, but only partial desensitization to SGN-00101. These results demonstrate that SGN-00101 is able to stimulate cells of the monocyte/macrophage lineage to secrete proinflammatory cytokines such as TNFα. Further, actions of SGN-00101 on THP-1 cells cannot be solely attributed to LPS as the stimulatory properties of SGN-00101 can be differentiated from those of LPS.

**P7** The Novel Adjuvant Om-174 Activates Murine Bone Marrow Dendritic Cells Through Tlr-4 And Can Also Signal Through Human Tlr-2

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1Department of Molecular Cell Biology, VUMC, Amsterdam, NETHERLANDS, 2Division of Immunology and Hematology, Kinderspital, Zurich, SWITZERLAND, 3OM PHARMA, Geneva, SWITZERLAND

OM-174, a novel adjuvant derived from lipid A (LA), stimulates murine dendritic cells (DC) in vivo. Lipopolysaccharide (LPS) and LA signal through TLR-4. The triacyl motif in OM-174 may act through TLR-4 but could also signal via TLRs not normally interacting with native LPS. Bone-marrow derived dendritic cells (BM-DC) from C3H/HeJ (TLR4 -/-) or C3H/OuJ (TLR-4 +/+ ) mice were stimulated with LPS (100ng/ml) or OM-174 (0.1-10mg/ml). Expression of CD86 and CD40 was assessed after 48h by FACS. HEK-293 cells, transfected with a human TLR-2 expression plasmid, were stimulated in culture with OM-174 (0.01-10mg/ml) or soluble bacterial lipopeptide (sBLP, 1mg/ml). Signalling through TLR-2 was detected by an NF-kB-Luc-reporter plasmid.

LPS and OM-174 increased expression of CD86 and CD40 in BM-DC from TLR-4 +/- mice. However TLR4 +/- mice did not increase CD86 expression, and only partially upregulated CD40. HEK-293 cells upregulated intracellular expression of NF-kB in response to OM-174 after transfection with a TLR-2 expression plasmid. These cells also responded to sBLP but not to purified LPS.

Conclusion: A functioning TLR-4 receptor is important in the response of BM-DC to OM-174, although other signalling systems may also play a role. Upregulation by OM-174 of NF-kB in transfected HEK-293 cells, suggests that it may also signal via human TLR-2. The potent responses to OM-174 in vivo may involve activation via more than one TLR.

**P8** The Development of Transgenic Plants for Production and Delivery of a Multicomponent Tuberculosis Vaccine

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Tuberculosis is the leading cause of death due to a single infectious agent among adults in the world. The current tuberculosis vaccine, bacillus Calmette-Guerin (BCG) has a variable protective efficacy and can cause serious and even fatal disseminated disease in immunocompromised patients. An effective, inexpensive, easily distributed and administered subunit vaccine is required for the control of tuberculosis. Recently, edible plants have been used successfully as production and delivery systems for viral, bacterial or mammalian antigens. Expression of vaccines in plant tissues eliminates the risk of contamination with animal pathogens, facilitates a heat-stable formulation, and enables mucosal delivery.

The purpose of our study is to evaluate the potential of a tuberculosis subunit vaccine based on two immunodominant antigens, Ag85B and the 6-kDa early secretory antigenic target (ESAT-6). In this study coding regions optimized for plant expression have been constructed for the antigens Ag85B and ESAT-6. These synthetic coding regions have been transcriptionally fused to the coding regions of a strong mucosal adjuvant to promote targeting to mucosal lymphoid tissues. The resulting coding sequences were cloned into plant expression vectors and transformed into three plant species using Agrobacterium-mediated transformation. PCR, ELISA and Western analysis were used to demonstrate the presence of the antigens of interest. Elite lines will be selected for use in future small animal challenge trials.
A Plant Based Breast Cancer Vaccine

1Department of Plant Biology, Arizona State University, Tempe, AZ, 2Boyce Thompson Institute for Plant Research, Ithaca, NY, 3Department of Biochemistry and Molecular Biology & Tumor Biology Program, Mayo Clinic Scottsdale, Scottsdale, AZ.

The American Cancer Society predicts that 203,500 women will be diagnosed with breast cancer this year. Despite progress in the treatment of cancer survival rates remain poor for patients with metastatic breast cancer. It has become evident that novel therapeutic approaches are necessary to advance patient care. The identification of tumor-associated antigens (TAAs), and their subsequent isolation, has revolutionized tumor immunology. Immunotherapeutic strategies or vaccination of patients using TAAs has been partially effective against some types of malignancies including B cell lymphoma and malignant melanoma. Plant biotechnology has been used in the past to create edible plant tissues containing genes derived from assorted pathogens (viral and bacterial). These “edible vaccines” have proven successful in animal challenge trials and human clinical trials. Using this technology, we have designed plant expression vectors that encode specific epitope sequences from known breast cancer TAAs, fused to the coding region of a known oral pathogen. Storage and delivery of a traditional vaccine is an expensive and unreliable procedure. Edible vaccines can be administered orally in rabbits and mice. Specific humoral immune responses when administered intraperitoneally or orally in rabbits and mice. These hantaviral recombinant nucleocapsid proteins were able to elicit specific humoral immune responses when administered intraperitoneally or orally in rabbits and mice.

Conclusion: The expression of the viral proteins in plants has major advantages compared to other expression systems: Firstly, there is no risk of contamination with mammalian viruses or other pathogens, and secondly, the production of high amounts of antigens is cheap and therefore of high economical interest.

Expression of Immunogenic Hantaviral Proteins in Transgenic Tobacco (Nicotiana tabacum) and Potato (Solanum tuberosum) Plants

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Background: Transgenic plants expressing foreign gene are suitable systems for the production of relevant immunogens in high amounts that can be used for development of new generations of vaccines against many infectious diseases.

Methods: The expression of nucleocapsid protein of hantavirus serotype Puumala in tobacco and potato plants was investigated. Transgenic tobacco (TNT-PUU-N) and potato plants (TST-PUU-N) were established. Results: These transgenic plants expressed the nucleocapsid protein of Puumala virus strain CG-1820. No major differences were observed when the phenotype and growth rates of transgenic plants were compared to that of normal plants. It was found that TNT-PUU-N and TST-PUU-N plants expressed the nucleocapsid protein in the leaves whereas TST-PUU-N plants express the viral proteins significantly also in the tubers and roots. The antigens were expressed at a level of 1 ng protein/5 mg dried leaves. Analogous experiments were performed with transgenic plants expressing hantaviral nucleocapsid proteins of the Hantaan serotype.

These hantaviral recombinant nucleocapsid proteins were able to elicit specific humoral immune response in fish when administered intraperitoneally or orally in rabbits and mice.

Conclusion: The expression of the viral proteins in plants has major advantages compared to other expression systems: Firstly, there is no risk of contamination with mammalian viruses or other pathogens, and secondly, the production of high amounts of antigens is cheap and therefore of high economical interest.

Expression of an SIV Protein in Transgenic Maize for Use as an Edible Vaccine and Reagent Supply

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There are several reports demonstrating that antigens derived from various pathogens can be synthesized at high levels in their authentic forms in plants. When administered orally by feeding, edible vaccines can induce an immune response and, in some cases, have shown to result in protection against a subsequent challenge with the pathogen. Storage and delivery of a traditional vaccine is an issue in developing countries due to problems such as lack of refrigeration. Many such problems can be alleviated using edible vaccines. Transgenic maize could be an excellent source of HIV-related proteins for edible vaccines as well as costly reagents. Toward these goals, we have transformed maize with an SIV protein gene. We have obtained expression of the protein in both transgenic callus and plants. Details of the system, including quantification of the protein in first generation seed, will be presented. NIH #1R21AI048374-01

CpG DNA and its Use in Fish Vaccines

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Bacterial DNA has a much higher unmethylated CpG dinucleotide content than vertebrate DNA. These ‘CpG Motifs’ have been shown to act as a ‘danger signal’ to the vertebrate immune system. However much of this work has been carried out on mammals. To date their effects on fish are poorly described. Here we report that CpG ODNs induce IL-1ß expression and production of interferon-like cytokines in head-kidney macrophages of rainbow trout (Oncorhyncus mykiss). Using a micro-chemotaxis chamber we found that CpGs act as a powerful chemo-attractant in fish in vivo. In addition, by i.p. injection of CpG and the collection of peritoneal cells we show that CpGs also induce a chemotactic response in vivo. However, this effect occurred at much lower concentrations in vivo than in vitro due to an influx of lymphocytes to the peritoneal cavity. CpG ODN were also found to have the ability to induce an innate immune response in fish later challenged with the furunculosis-causing bacteria Aeromonas salmonicida.

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P13 Age Difference in Lymphocyte Proliferation, and IL-2 and IFN-gamma Production Following Salmonella Enteritidis Vaccination

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The present study was conducted to investigate the effect of age on cell-mediated immune responses to different antigens from Salmonella enterica Enteritidis (SE) following vaccination with the commercially available heat-killed bacterin. Eight-month- and 4-week-old chickens were given two subcutaneous injections with SE bacterin at 2 weeks apart. At 4, 7, 11, 14, 18, and 21 days post immunization (PI), spleen lymphocytes were stimulated with Concanavalin (Con A), heat-killed SE (HK-SE), LPS, flagella, outer membrane protein (OMP), and porin and their proliferation measured by a non-radioactive method using a tetrazolium salt, WST-8. Interferon (IFN-gamma) and interleukin2 (IL-2) produced by spleen cells stimulated with different SE antigens and serum cytokine levels were assessed using an ELISA specific for cytokines. Vaccinated chickens of 4-week of age showed higher proliferation response to LPS and flagella antigen, but not to the other antigens, compared to the unvaccinated chicken, whereas 8-month-old chickens showed a reduced proliferation response to SE antigens. Serum IFN-gamma and IL-2 levels were higher in the vaccinated birds compared to the age-matched control regardless of the age group. The levels of IFN-gamma produced by the SE antigen-stimulated spleen cells were higher in the vaccinated young birds compared to the old chickens. These results indicate that various antigenic components of SE bacteria induce IFN-gamma and IL-2 production, and younger chickens show better T-lymphocyte-mediated immunity following SE vaccination.

P14 High incidence of Coccidioides immitis seroconversion in dogs points to need for canine vaccine trials

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Coccidioidomycosis (CI) is prevalent in dogs from endemic areas and may lead to serious disease. As a part of our human vaccine effort, we performed a pilot study to determine incidence and clinical disease of CI among dogs as a prelude to vaccine field trials in dogs. Young dogs presenting for routine veterinary care were recruited from clinics in Tucson and were evaluated with cocci serology and skin testing, exam, and an environmental questionnaire. In the first 6 months of followup 74 dogs aged 4-8 months were screened; 67/4 were sero-positive at baseline; an additional 2 dogs were excluded because of unrelated illness. Of 66 seronegative dogs followed over 6 months, 6 seroconverted, one of whom became clinically ill. Only 2/6 serologically positive dogs were also skin test positive. These data indicate a 9.0% (95% CI 3.4%-18.7%) incidence over 6 months for seronegative dogs, or a combined incidence of 25.2% per year for all dogs, including those positive at baseline. The incidence of clinical disease was 1/66 over 6 months, or 3% per year. These data indicate that incident infection with CI is high in young dogs and are similar to those among humans in endemic areas. However, clinical disease was relatively uncommon during this initial followup. Further evaluation over a longer period will likely reveal more clinical disease. An effective canine vaccine may have high marketability.

P15 Mycobacterium bovis BCG Vaccination of Cattle: Activation and Proliferation of Lymphocyte Subsets Upon Stimulation With Mycobacterial Antigens

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Efforts to eradicate *Mycobacterium bovis* infection from cattle within the United States are hindered by the emergence of a wildlife reservoir (i.e., white-tailed deer) and the importation of tuberculous cattle from Mexico. Vaccination may become necessary for control of bovine tuberculosis. To determine proliferative responses and activation marker expression on lymphocytes stimulated with mycobacterial antigens, peripheral blood mononuclear cells from *M. bovis* BCG-vaccinated cattle were stained with the green fluorescent dye, PKH67, and cultured (2–8d) with or without soluble mycobacterial antigens (i.e., PPD or WCS). Cells were harvested and evaluated by flow cytometry for proliferation (PKH67), activation (CD25, CD44, CD62L), and phenotype (CD4, CD8, gd TCR+) at various time points during the incubation period. Data were analyzed as a split-plot ANOVA.

Fisher’s protected-LSD test was applied when treatment effects (P<0.05) were detected by the model. Both CD4+ and gd TCR+ T cells but not CD8+ T cells from vaccinated cattle compared to cells from non-vaccinates proliferated (p<0.01) to soluble *M. bovis* antigens. The median fluorescence intensity of CD25 and CD44 increased whereas CD62L decreased (p<0.05) on proliferating (i.e., PKH67 dim) CD4+ and gd TCR+ cells as compared to non-proliferative fractions (i.e., PKH67 bright) of the respective subsets. Such alterations in cytokine receptor and adhesion molecule expression would impact trafficking and functional capacities of T cells proliferating in response to mycobacterial antigens.

P16 Humoral Response of Nelore Calves After Application of Autovaccines Prepared From Pseudomonas Aeruginosa

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**Background:** Specific autovaccines (prepared as a whole cell therapeutic vaccine from cultured bacteria found to be responsible for a given disease) are used on a regular basis in veterinary medicine for the treatment of both viral and bacterial infections. Although efficacious there is only little knowledge about the induction of effector mechanisms by autovaccines.

**Methods:** Thirteen 10 weeks old Nelore calves each were artificially infected at different sites with *P. aeruginosa* to mimic an infection (Otitis media, sinusitis and urinary tract infection, respectively). On day seven post infection 5 animals of each group received subcutaneous autovaccines prepared from the *P. aeruginosa* strain which was used for infection, 5 animals each the autovaccine per oral and subcutaneous and three animals each served as a control, remaining non vaccinated. Serum samples were taken immediately prior to infection and four times following start of autovaccination. Immunoglobulin titers were measured in a serum gel precipitation assay.

**Results:** The control animals which were infected but not vaccinated showed on an average less than a twofold increase in specific antibodies over a three week period. Those animals who received autovaccination showed an 14-fold to 60-fold increase in specific antibodies. The increase differed after subcutaneous or subcutaneous/oral application only in the Otitis media group.

**Conclusion:** These data suggest that autovaccines made from *P. aeruginosa* induces specific antibodies, regardless of the application route.
P17 Immune responses in vivo.

Progress to delineate the role of LCs in antigen presentation and induction of immunization site within 48 hours. Antigen containing LCs originating at the immunization site was detected within 2-4 hours of EPI and nearly all EPI intrinsically induced activation and migration of LCs. LC activation at the junction. Presence of Texas Red-labeled antigen in the LCs of the epidermis and sugar particles were located in the epidermis and the epidermal-dermal device. Histological examination of the target site showed that both the gold coated on the surface of gold micro-particles (1-2 mm) or embedded in the epidermis, are important in the immune responses to EPI. Antigens were determined if Langerhans cells (LCs), potent antigen presenting cells in the bacterial and viral antigens in mice. The present studies were performed to determine if LCs, potent antigen presenting cells in the epidermis, are important in the immune responses to EPI. Antigens were coated on the surface of gold micro-particles (1-2 mm) or embedded in the sugar excipient particles (20-50 m m). These formulations were administered to the mouse ear or abdominal skin using a needle-free powder delivery device. Histological examination of the target site showed that both the gold and sugar particles were located in the epidermis and the epidermal-dermal junction. Presence of Texas Red-labeled antigen in the LCs of the epidermis was confirmed by immunohistochemistry assays. Further studies showed that EPI intrinsically induced activation and migration of LCs. LC activation at the immunization site was detected within 2-4 hours of EPI and nearly all LCs, many of them containing antigen, migrated away from the immunization site within 48 hours. Antigen containing LCs originating at the vaccination sites were detected in the draining lymph nodes 24 hours after immunization. Studies using isolated LCs from the immunization sites are in progress to delineate the role of LCs in antigen presentation and induction of immune responses in vivo.

P18 Antibody Response to Vaccination With Inactivated Cowdria Ruminantium (gardel Strain) in Ugandan goats

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Cowdriosis (heartwater), caused by *Cowdria ruminantium* and transmitted largely by *Amblyomma* ticks, is endemic in much of Uganda and affects cattle, goats and sheep. This study examined the effectiveness of vaccination in controlling Cowdriosis in goats. Seventy (70) goats of mixed breeds, with no antibody titres against *Cowdria* were immunized. Each animal was injected with 250 mg of inactivated elementary bodies of *C. ruminantium* (Gardel strain) and boosted two weeks later. After 14 days, the goats were exposed to natural tick challenge. Sera were evaluated weekly by ELISA for the presence of *C. ruminantium* specific antibodies. Vaccinated goats developed *C. ruminantium* antibodies. Control animals injected with only adjuvant remained sero-negative, although those subjected to the natural field challenge also developed antibodies. There was a statistically significant difference (P < 0.001) in mean response between the vaccinated and control animals. There was no significant difference in the individual weekly variation of the mean O.D between days 0 to day 35. A significant difference (P < 0.001) between responses was noted from days 42 to 133 with the vaccinated animals having higher antibody response. There was a significant difference (P < 0.034) in mortality rates among vaccinated animals (0%) compared to control animals (11.1%). The results indicate that this vaccine can possibly protect the goats under field conditions.

<table>
<thead>
<tr>
<th>Day Post infection</th>
<th>Percentage Positivity of Goats immunized with vaccine</th>
<th>Percentage Positivity of Goats immunized with adjuvant only</th>
<th>Day Post infection</th>
<th>Percentage Positivity of Goats immunized with vaccine</th>
<th>Percentage Positivity of Goats immunized with adjuvant only</th>
</tr>
</thead>
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<tr>
<td>Day 0</td>
<td>0%</td>
<td>0%</td>
<td>Day 49</td>
<td>100%</td>
<td>87.5%</td>
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<tr>
<td>Day 7</td>
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<td>0%</td>
<td>Day 56</td>
<td>100%</td>
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<td>Day 14</td>
<td>89.3%</td>
<td>0%</td>
<td>Day 71</td>
<td>96.4%</td>
<td>70.8%</td>
</tr>
<tr>
<td>Day 21</td>
<td>96.4%</td>
<td>0%</td>
<td>Day 90</td>
<td>100%</td>
<td>63%</td>
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<tr>
<td>Day 28</td>
<td>96.3%</td>
<td>0%</td>
<td>Day 115</td>
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<tr>
<td>Day 35</td>
<td>100%</td>
<td>0%</td>
<td>Day 125</td>
<td>96.4%</td>
<td>33.3%</td>
</tr>
<tr>
<td>Day 42</td>
<td>96.2%</td>
<td>62%</td>
<td>Day 133</td>
<td>92.9%</td>
<td>58.3%</td>
</tr>
</tbody>
</table>

P19 Role of Langerhans Cells in Epidermal Powder Immunization

D. Chen, Q. Chu, L. G. Payne
PowderJect Vaccines, Madison, WI

We have previously shown that epidermal powder immunization (EPI) induces robust cellular and humoral immune responses to a variety of bacterial and viral antigens in mice. The present studies were performed to determine if Langerhans cells (LCs), potent antigen presenting cells in the epidermis, are important in the immune responses to EPI. Antigens were coated on the surface of gold micro-particles (1-2 m m) or embedded in the sugar excipient particles (20-50 m m). These formulations were administered to the mouse ear or abdominal skin using a needle-free powder delivery device. Histological examination of the target site showed that both the gold and sugar particles were located in the epidermis and the epidermal-dermal junction. Presence of Texas Red-labeled antigen in the LCs of the epidermis was confirmed by immunohistochemistry assays. Further studies showed that EPI intrinsically induced activation and migration of LCs. LC activation at the immunization site was detected within 2-4 hours of EPI and nearly all LCs, many of them containing antigen, migrated away from the immunization site within 48 hours. Antigen containing LCs originating at the vaccination sites were detected in the draining lymph nodes 24 hours after immunization. Studies using isolated LCs from the immunization sites are in progress to delineate the role of LCs in antigen presentation and induction of immune responses in vivo.

P20 Amino Acid Dimorphism in the Merozoite Surface Protein-1 of Plasmodium Falciparum as Immune Evasion Mechanism

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Proteins of protozoan parasites exposed to the immune system display high levels of sequence polymorphism. The N-terminal block of MSP-1, a leading malaria vaccine candidate, contains three dimorphic amino acid positions (amino acids 44, 47 and 52) and six combinations of these three dimorphic positions (S44–Q47–V52, S44–H47–I52, G44–H47–I52, G44–Q47–V52, G44–H47–I52 and G44–H47–V52) have been found in P. falciparum populations worldwide. This type of diversity is presumed to be associated with parasite immune evasion representing one major obstacle to vaccine development.

In order to investigate the influence of amino acid dimorphism on cellular immune responses, a series of CD4 T cell lines and clones specific for the S44–Q47–V53 allelic variant of the MSP-138-58 epitope were established from several donors immunized with the peptide vaccine SP66 and tested for MHC restriction and cross-reactivity with allelic sequence variants in proliferation assays and cytokine secretion analysis.

The human MSP-138-58 specific T cell lines and clones were restricted by HLA-DR and -DP molecules. T cells were exclusively specific for the sequence variant used for immunization. Peptide binding assays with affinity purified HLA-DR and -DP molecules. T cells were exclusively specific for the sequence variant used for immunization. Peptide binding assays with affinity purified HLA-DR and -DP molecules. T cells were exclusively specific for the sequence variant used for immunization. Peptide binding assays with affinity purified HLA-DR and -DP molecules. T cells were exclusively specific for the sequence variant used for immunization. Peptide binding assays with affinity purified HLA-DR and -DP molecules. T cells were exclusively specific for the sequence variant used for immunization.
Evaluation of high molecular weight polysaccharide adhesin from Staphylococcus aureus.

J. Joyce¹, C. Abeygunawardana¹, Q. Xu¹, J. Cook¹, R. Hepler¹, C. Przysiecki¹, K. Grimm¹, K. Roper¹, C. Ip¹, L. Cope¹, D. Montgomery¹, M. Chang¹, S. Campie¹, G. B. Pier², G. Mark¹, P. Keller¹, K. Jansen¹

¹Merck Research Laboratories, West Point, PA, ²Harvard Medical School, Brigham and Women’s Hospital, Boston, MA

In an effort to develop a vaccine for staphylococcal infections, we have purified a high molecular weight S. aureus exopolysaccharide (SAE), characterized its structure, and evaluated its antigenicity in mice. SAE was purified from S. aureus by tangential flow filtration and ion-exchange chromatography. Purified SAE was sonicated to generate a sized polysaccharide. NMR and HPAEC analyses were used to determine composition and structure. Native and sized materials were covalently coupled to Neisseria meningitidis OMPC and used to vaccinate mice for evaluation of potential immune response.

SAE is a polymer of β-1,6 glucosamine with approximately 50% N-acetyl substitution and 10% O-succinate. No evidence of N-succinylation was found. NMR analysis demonstrated that previous literature reports of N-succinate were due to an artifact of sample preparation. SAE was of high MW (> 300,000 Da), and chemical treatment with HF or sonication reduced the MW to < 100,000 Da. Both forms of SAE were capable of agglutinating sheep red blood cells, but HF-treated SAE was less effective. Immunization of mice with both native and sized SAE conjugated to OMPC elicited high antigen-specific titers in mice.

SAE isolated from S. aureus is a high MW β-1,6-linked homopolymer of glucosamine variably substituted with N-acetate and O-succinate. It is very similar chemically to the polysaccharide intercellular adhesin (PIA) isolated from S. epidermidis.
Virus Based Vaccines for Cancer Therapy
J. D. Nieland
Practical Development, Medigene AG, Munich, GERMANY

Viruses can induce tumor specific immune responses by different mechanisms:
1. Viruses induce tumor lysis and thereby release of tumor antigens;
2. Recombinant viruses introduce immune stimulatory molecules into tumor cells;
3. Recombinant viruses or genome free chimeric virus-like particles (CVLPs) introduce tumor antigens to the immune system.

Medigene develops products based on all three strategies. Two of these projects are HSV-1 and HPV16 CVLP.

Modified HSV-1 viruses that only replicate within tumor cells and destroy them through oncolysis (G207 and NV1520), are tested in clinical trials (colon carcinoma, phase I; brain tumor, phase II). During oncolysis tumor antigens enter the immune system which after uptake by APC stimulate a tumor specific helper and cytotoxic T cell responses.

Human papillomavirus type 16 CVLPs are currently tested in a phase I clinical trial as therapeutic vaccines for the treatment of high grade CIN patients. The CVLPs consist of HPV 16 L1E7 fusion proteins. L1 is the major virus capsid protein, whereas E7 is constitutively expressed in virus infected cells and regarded a tumor antigen. CVLPs are able to pseudo-infect and activate pre-dendritic cells. The pseudo-infected activated dendritic cells are recognized by circulating L1- and E7-specific CTLs. This could be shown in vitro with PBL of healthy donors and in vivo in mice. These CTLs are able to recognize and lyse antigen-expressing target cells, and to protect mice from the outgrowth of E7-expressing tumor cells.

In summary these projects seem to be feasible in respect to production and therapeutic application, and promising so far from a clinical point of view.

HSV Vectors for Cancer and Infectious Disease Immunotherapy
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1Biovax Ltd, Abingdon, UNITED KINGDOM, 2University College London, London, UNITED KINGDOM

HSV infects DC efficiently but with minimal replication, suggesting that the lifecycle of HSV usually includes the infection of DC, eventhough DC are non-permissive for virus growth. It also provides the potential to use HSV as an efficient means of gene delivery to DC in immunotherapy. However, DC infected with HSV usually loose the ability to become activated. We have found that the virion host shut off protein (vhs) plays a key role in this inactivation process. DC infected with disabled HSV vectors from which the vhs protein has been deleted retain functionality and become activated, rather than having activation blocked in response to infection or other stimuli. Optimised HSV vectors have been generated in which vhs has been deleted in combination with ICP47, previously identified as inhibiting antigen presentation in HSV infected cells. The vectors described, unlike wild-type HSV, significantly up-regulate levels of CD80, CD83, CD86, CD40 and MHC class I on infected cells, give very high level transduction of DC at a low virus dose (MOI=1), and have a potent immune-stimulating capacity. Vectors are in development for the treatment of melanoma via the delivery of multiple melanoma antigen-genes following ex vivo delivery to DC, and for the treatment of infectious diseases via the delivery of appropriate antigens directly in vivo.
**P29** Is Crohn’s Disease Treatable with Therapeutic Autovaccines?  
1Hygiene-Institut, Heidelberg, GERMANY, 2Solitude Klinik, Ludwigshafen, GERMANY

**Background:** The aetiology of Crohn’s disease, an inflammatory bowel disease, is still unclear. There is still debate about whether bacteria (i.e. Mycobacterium paratuberculosis) or virus (i.e. HHV6) are -at least in addition to nutritional allergies- responsible for this disease. The purpose of our study was to find out whether autovaccines manufactured from tissue biopsies and positive for HHV6 would be an alternative for the treatment of Crohn’s disease.

**Methods:** In collaboration with a clinic in Ludwigshafen (Germany) we have manufactured and applied patient autovaccines for 9 patients who suffered Crohn’s disease since several years in different degrees of severity. For preparation of autovaccines usually 20 to 30 biopsies were taken. The biopsies were transferred into sterile endotoxin free PBS and washed twice. The washed specimens were processed according to the description given in DE 100 21 433-A1. The autovaccines (prepared without further cultivation of microorganisms) were applied subcutaneous as 2 mL doses once a week over four weeks. All patients were advised to follow a special diet.

**Results:** HHV6 DNA was detected in biopsy specimens taken previous to autovaccination from the intestine of all patients. Patients who complied to their diet showed improvement in their condition following autovaccination. Three patients who did not comply to the dietary recommendations displayed no or little improvement. Our conclusion from this preliminary experiment is that therapeutic autovaccination in combination with specific diet is an alternative treatment of Crohn’s disease which should be further examined, at least in cases where intestinal biopsies are positive for HHV6.

**P30** Factors associated with (lack of) influenza vaccination among health care workers in Germany  
U. Buchholz  
Infektionsepidemiologien, Robert Koch Institute, Berlin, GERMANY

**Background:** The Standing Committee for Vaccination Practices in Germany recommends that HCWs be vaccinated against influenza. In preparation for an intervention program to increase vaccination coverage among HCWs we conducted a survey to get baseline information from the season 2001/2002.

**Methods:** To 34 hospitals from the German national surveillance system for nosocomial infections (KISS) we sent one questionnaire for the hospital infection control specialist (questionnaire 1), and 40 anonymous questionnaires to HCWs (questionnaire 2). HCW were selected through systematic sampling. We used multivariate regression to identify factors associated with vaccination.

**Results:** Questionnaire 1: 22 (65%) of them had recommended the influenza vaccine to their medical personnel, 23 (67%) offered influenza vaccination to their staff free of charge. Questionnaire 2: We received 886 questionnaires, i.e. 26 completed questionnaires per hospital. Only 383 (44%) HCWs believed that they were at increased risk to contract influenza, and only 301 (35%) thought that the influenza vaccine was highly effective. 131 (15%) were vaccinated against influenza in the season 2001/2002 and 94 (72%) of them had also been vaccinated in the season 2000/2001. Influenza vaccination in the previous year and the belief that the vaccine is highly effective were the best predictors for having received an influenza vaccine.

**Conclusions:** Influenza vaccination rates continue to be extremely low among German HCWs. An intervention program should emphasize free influenza vaccination in hospitals, but above all must inform medical personnel about their risk to contract influenza, the effectiveness of the vaccine, and their responsibility to protect their patients through vaccination.

**P31** Hypersensitivity to Thimerosal in the Skin-test Antigen Coccidioidin  
1Epidemiology & Biostatistics, UCSF, San Francisco, CA, 2Section of Infectious Diseases, Naval Medical Center, San Diego, CA, 3Section of Infectious Diseases, S. Arizona VAHSC, Tucson, AZ

Coccidioidin is an important epidemiologic tool that will be utilized for the screening of prospective vaccines for planned coccidioidomycosis vaccine trials. During the course of a Phase 1 study of this skin-test antigen in subjects with documented prior coccidioidomycosis, a high percentage of hypersensitivity to thimerosal (1:10,000 w/v) vehicle control was encountered, with DTH reactions in 11/14 subjects. The contribution of hypersensitivity reaction to thimerosal rendered the interpretation of the coccidioidin reaction nugatory. Coccidioidin was reformulated with a phenol preservative but still contained trace thimerosal (up to 1:250,000 w/v) derived from the original coccidioidin concentrate. Consequently, a vehicle control containing a similar quantity of thimerosal was included for a second study. In 20 subjects enrolled, 5 had measurable indurations of any size at 24 or 48 h to the trace-thimerosal vehicle, including one reaction of 12x11 mm. Overall, the results suggest that the previous literature on thimerosal hypersensitivity may be an underestimate of the prevalence of reactors and that data generated with any skin-test antigen containing this preservative must control for this reaction.

**P32** Parents Want to be Offered the Varicella Vaccine Whatever Their Physician’s Opinion  
G. De Serres, L. Rochette, B. Duval, N. Boulianne  
INSPQ, Quebec, PQ, CANADA

**Background:** In Quebec City in 2000, a previous study found that only 37% of parents attending the 12 month visit with their child were offered the varicella vaccine by their provider. The objective was to assess if parents wanted to be offered the vaccine even if their physician considers the disease too benign to be worth prevented or the vaccine too expensive.

**Methods:** 663 parents of children aged from 14 to 17 months were phone interviewed. **Results:** Overall 96% wanted to be informed on varicella vaccine independently of their physician’s personal opinion about it, although most also wanted his opinion. 89% of parents wanted to be offered the vaccine even if their physician considers the vaccine too expensive. 94% of parents who did not want their physician to offer the vaccine was higher among parents with low household income (<40,000$Can) than those with a higher one (12% vs 7%). 80% of parents wanted to be offered the vaccine by their physician even if he considers varicella too benign to be worth prevented.

**Conclusion:** Parents want to be offered the varicella vaccine whatever their physician’s personal opinion on the severity of varicella or the cost of the vaccine.
**P33** The Postmarketing Safety Review of Reports of Herpes Zoster After the Administration of VARIVAX® [Varicella Virus Vaccine Live (OKA/MERCK)]

S. Gale1, A. Sweet1, A. Gershon2, P. LaRussa2, S. Steinberg2, S. Music1, R. Sharrar1
1Merck & Co. Inc., West Point, PA, 2Columbia University College of Physicians and Surgeons, New York, NY

Herpes zoster (HZ) has been reported following wild-type (WT) varicella, and after vaccination with VARIVAX®. HZ results when varicella zoster virus (VZV) reactivates from latency in dorsal root ganglia. This review includes an epidemiologic analysis of reports to Merck & Co., Inc. of HZ occurring after vaccination with VARIVAX®. Following the spontaneous reporting of HZ after VARIVAX® administration, cases were retrospectively reviewed. In collaboration between Merck & Co., Inc. and Columbia University, specimens were analyzed by polymerase chain reaction (PCR) to identify the presence of either WT VZV or Oka/Merck vaccine VZV. PCR analysis does not establish causation.

In the six-year postmarketing period, there were 381 physician-diagnosed HZ reports, from 2-2021 days postvaccination, in patients 1-68 years of age, 71% in patients <5 years old. Of 104 specimens, the presence of Oka/Merck VZV was identified in 35 (34%) and WT VZV in 26 (25%); 3 (3%) specimens were positive for VZV but were unable to be typed. 9 (8%) specimens were negative for VZV and 31 (30%) specimens were inadequate.

The overall reporting rate of physician-diagnosed HZ was 1.4 cases per 100,000 doses of VARIVAX® distributed (381/281.1 million doses). This compares to an incidence of 110 HZ cases per 100,000 persons <5 years old with prior varicella infection. (1)


**P34** Designing Trials to Detect Herd Immunity Effects For NonTypeable Haemophilus influenzae Vaccines

J. S. Koopman1, X. Lin2
1Epidemiology, University of Michigan, Ann Arbor, MI, 2Michigan, A2, MI

Background: The herd immunity effects of Hib vaccines indicate that trials should be designed to detect transmission effects of NonTypeable Haemophilus influenzae (NTHi) vaccines. The type and degree of immunity stimulated by NTHi NP infection is poorly known. We developed models to infer individual and herd immunity effects of natural infection. Methods: A deterministic compartmental model of the NTHi transmission system was constructed. A multistage process was set for NP infection and oritis media. Separate immunity effects on susceptibility, contagiousness, duration, and pathogenicity of NP infection were formulated. Model parameters were fit to observed disease levels.

Results: Observed patterns of infection and oritis could be fit only when each infection stimulated considerable immunity to susceptibility, contagiousness, and pathogenicity and when additional immunity was acquired after each of at least four infections. Even though daycare attendance tripled infection rates, infection levels in other groups were insensitive to changes in daycare transmission. The high rate of infection required to fit observed data caused vaccination effects on transmission to be quickly wiped out if high levels of vaccination were not maintained.

Conclusions: The strong immunity found for natural NP infection is encouraging for vaccine development. Antigenic diversity likely explains the continued acquisition of immunity with multiple infections. The insensitivity of infection levels within daycare centers to outside infection levels should facilitate the design of trials to detect herd immunity effects. But sustaining herd immunity effects for NTHi will require much more intensive and repeated immunization than was the case for Hib.

**P35** Promoting Public/Private Partnerships: A Model of Public Health Participation in Clinical Trial Research

S. J. Marks, MPA1, L. Squires1, P. Barrett1, M. C. Caldwell, MD, MPH1
1Communicable Disease Control Division, Dutchess County Department of Health, Poughkeepsie, NY, 2Dutchess County Department of Health, Poughkeepsie, NY

Background: CDC, state and local governments promote public/private partnerships. This presentation illustrates mutually advantageous strategies enabling clinical study sponsors and local public health to strategically place studies based on epidemiologic data. The primarily passive role of public health in pharmaceutical research profoundly confines its ability to establish a comprehensive approach regarding the changing dynamics of health care.

Methods: Clinical study sponsors express desire for some level of public health involvement or support. We present compelling issues motivating collaboration between public health and study sponsors, e.g., newly emerging pathogens and antimicrobial resistance; vaccine development/advocacy; selection of study sites having epidemiological significance; novel, “cutting edge” disease intervention; increased provider/public awareness of local disease morbidity/trends; academic prevention/early disease detection; bioterrorism response. Perceived misconceptions regarding public health involvement, e.g., “conflict of interest” and liability) are explored.

Results: Placement of studies that are of local epidemiologic importance has increased acceptance of clinical research among local medical providers and community-at-large. Participation in studies has enhanced our ability to meet traditional public health mandates and gain support from community advocates/groups.

Conclusions: The presentation presents a model of public health participation in clinical research. The current political and health care environment requires public health flexibility in addressing traditional mandates and emerging issues, such as bioterrorism, antimicrobial resistance, prevention and disease intervention, especially enhancing access to healthcare for traditionally underserved populations.

**P36** Volunteer Recruitment for Phase 1 HIV Vaccine Clinical Trials in Nairobi, Kenya

G. S. O. M. Omosa-Manyonyi, Sr.
Medical Microbiology - Kenya AIDS Vaccine Project, University of Nairobi, Nairobi, KENYA

Background: HIV is spreading rapidly with over 90% of the new infections occurring in developing countries. In Kenya alone there are about 700 new infections every day. There is therefore need to explore other approaches to control the HIV epidemic such as a preventive vaccine.

The HIV vaccine undergoing Clinical Trials in Kenya is of the clade A subtype which is the prevalent HIV sub-type in this region. The study is double-blind and placebo controlled involving 18 healthy HIV-1&2 uninfected adult volunteers with a low risk of HIV-1&2 infection.

Volunteer recruitment methods:
- Advertisements/Sensitization seminars
- Information Seminars/Sessions
- Screening (Medical and social history, physical examination, chest X-ray, laboratory tests)
- Enrollment of eligible volunteers.

Results:
- 43 volunteers were screened and 19 were eligible but one declined enrolment
- Of those not eligible the reasons were laboratory abnormalities
- Most of those eligible were below 40 years of age, single (67%), and college students
- Male to female ratio of enrollees was 15:3
- 50% of the enrolled volunteers did not want their family members to know about their participation in the trial

Conclusions:
- Most people are willing to support the vaccine trials through volunteering and otherwise
- The decision to participate is a personal affair and confidentiality must therefore be maintained
- We need to re-strategize our recruitment process for future trials so as to get a more varied group of volunteers e.g. more female participants.

Acknowledgements:
- University of Nairobi
- International AIDS Vaccine Initiative
- Medical Research Council
P37 Considerations for Future HAV Vaccine Development
G. Raychaudhuri
FDA, Rockville, MD

Hepatitis A causes significant morbidity worldwide, and continues to be one of the most vaccine-preventable diseases in the United States. Hepatitis A virus (HAV) is transmitted primarily by the fecal-oral route, and epidemics are common in regions where sanitation conditions are poor. Currently available methods of prophylaxis against hepatitis A include passive immunization with immune globulin or active immunization with inactivated HAV vaccines. The recently licensed inactivated HAV vaccines have proven to be safe and highly efficacious, and provide long-term immunity against hepatitis A disease. Implementation of a number of public health measures would facilitate more effective control of hepatitis A. These include widespread immunization of young at risk children who are effective vectors for transmission of the virus, post-exposure prophylaxis in outbreak situations, and implementation of a practical and affordable vaccination strategy for developing countries, where most cases of hepatitis A occur. Future efforts in HAV vaccine development should address these fundamental public health needs.

A number of advancements in HAV vaccine development could help to achieve these goals. These include:
• Evaluation of HAV vaccines for efficacy in post-exposure prophylaxis to control spread of HAV in outbreak situations.
• Inclusion of HAV in new combination vaccines, which could facilitate higher compliance for HAV immunization.
• Extension of the indication to younger age groups.
• Development of safe and effective live, attenuated HAV vaccines which could make large scale vaccination of populations more feasible, especially in developing countries.
• The advantages and disadvantages, as well as the regulatory considerations for these approaches to future HAV vaccine development will be discussed in the presentation.

P38 BCG Scar and Positive Tuberculin Reaction Associated with Reduced Child Mortality in West Africa. A Non-specific Beneficial Effect of BCG?
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1Department of Epidemiology Research, Statens Serum Institut, Copenhagen, DENMARK, 2Projecto de Saúde, Bandim, Bissau, GUINEA-BISSAU, 3Medical Research Laboratories, Fajara, GAMBIA

Background: Previous studies have suggested that the BCG vaccine may have a non-specific beneficial effect on childhood survival in areas with high mortality. We therefore examined whether BCG-vaccinated children who developed a BCG scar or a positive tuberculin reaction had better survival than children who did not develop such reactions.

Methods: We examined 1,813 children for BCG scar at 6 months of age and 813 BCG-vaccinated children were skin-tested for delayed hypersensitivity to tuberculin, tetanus and diphtheria.

Findings: BCG-vaccinated children with a BCG scar had significantly lower mortality compared with BCG scar-negative children, the mortality ratio in the first 12 months being 0.41 (0.25-0.67). BCG-vaccinated children with a positive tuberculin test had a mortality ratio of 0.45 (0.24-0.85) compared with tuberculin negative children. After censoring for tuberculosis exposure at home, the mortality ratios for having a scar and being tuberculin-positive were 0.46 (0.27-0.79) or 0.42 (0.21-0.84), respectively.

Interpretation: Response to BCG vaccination as measured by a BCG scar or a positive tuberculin reaction was associated with better survival in early childhood in an area with high mortality. Since nothing similar was found for responders to diphtheria-tetanus-pertussis vaccine, and the effect could not be explained by protection against tuberculosis, it could be due to non-specific immune-stimulation protecting against other infections.

WITHDRAWN
**P41 Life, History and Health Predicts Vaccine-induced Cytokine Production**

**M. S. Hayney¹, G. D. Love¹, J. M. Buck², D. Muller¹**

¹University of Wisconsin, Madison, WI, ²University of Minnesota, Minneapolis, MN

**Background:** Existing data suggest that many aspects of immune function are compromised by poor psychological well-being or social relationships. We hypothesized that high psychological well-being and high quality relationships would be associated with vigorous cell mediated immune responses to vaccination.

**Methods:** Eighty-nine individuals were immunized with influenza and hepatitis A vaccines. Blood was drawn for antibody measurements and measurements of cytokine production as an indicator of cell mediated immunity prior to and 14 and 28 days after immunization. Psychosocial characteristics were assessed using the Psychological Well-Being scale, the Parental Bonding scale and the Personal Assessment of Intimacy in Relationships (PAIR, to assess spousal relationship). Correlations were made between measures of psychological well-being and quality relationships and cytokine production.

**Results:** Interleukin-10 (IL-10) production on day 28 was statistically significantly increased over baseline and day 14 for both influenza and hepatitis A. Interferon g (IFNg) production on day 28 was statistically significantly greater than baseline following hepatitis A immunization. Significant positive correlations were made between psychological well-being and quality relationships and IL-10 and IFNg production on day 28. In addition, the association was improved when the model included both psychological well-being and quality relationships.

**Conclusions:** High psychological well-being and quality relationships are associated with vigorous cell mediated immune responses following immunization. This study represents one of the first to show positive physical health is associated with psychological well-being and quality relationships.

**P42 Establishment of Minimum Protective Threshold Serum Antibody Titer to Respiratory Syncytial Virus (RSV) Associated Hospitalization (RSV-AH)**

**P. A. Piedra¹, A. M. Jewell¹, S. G. Cron², W. P. Glezen¹, P. W. Hiatt²**

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RSV is an important respiratory pathogen among all ages. Subunit and live RSV vaccines are in development. Although immunity to RSV is incomplete, RSV-specific serum antibodies play a role in preventing significant disease.

Establishment of a protective threshold titer will aid vaccine development. From June 1991 to June 1993 participants enrolled in a prospective trial to determine the frequency of virus specific infections associated with hospitalization were eligible for this analysis. 184 of 532 individuals 0.1-89 years of age met criteria; virus culture and acute blood sample at hospitalization and convalescent blood sample 2 to 8 weeks later. RSV infection was defined by a positive culture and/or serology (microneutralization to RSV/A or RSV/B, ELISA to fusion (F) protein, or Western blot assay). RSV-AH occurred in 11 of 29 infants (< 1 year), 8 of 21 young children (1-4 years) and 17 of 134 older children and adults (> 5 years). At hospitalization neutralizing antibody titers to RSV/A and RSV/B, and binding antibody titer to F protein were lower in patients with RSV-AH (p < 0.01). For every log increase in titer there was - 20% increase in the likelihood of not having an RSV-AH. Using quartile values, a minimum protective neutralizing antibody titer (log2) of ≥ 6.5 to RSV/A (OR 3.2 CI 1.4-7.7) and ≥ 8.0 to RSV/B (OR 2.8 CI 1.1-6.9) was established against RSV-AH.