

# on Vaccine Research

## ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

### **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<sup>-6</sup> PFU/ml with a total energy of 3.7 J/cm<sup>2</sup> and 4.2 J/cm<sup>2</sup> (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<sup>6</sup> pfu HSV2 and monitored 50d 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**

**WITHDRAWN**

### **P4** 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 codelivery of pE and nonstructural gene plasmids can enhance anti-E antibody titers, interferon-gamma production, and cytotoxic 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.

P5

WITHDRAWN

### 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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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 (TLR4 +/-) 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.

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<sup>+</sup> 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 LPS, SGN-00101 or its 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. TNFa production was assessed by ELISA.

HspE7, but not Hsp65, E7 (or the admixture) induced the release of TNFa from THP-1 cells. While SGN-00101 was able to elicit TNFa release from THP-1 cells cultured in low serum containing media, LPS did not elicit TNFa 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 TNFa. 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.

### 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.

# on Vaccine Research

## ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

**P9** **A Plant Based Breast Cancer Vaccine**  
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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 adjuvant. We have used these plant expression vectors to transform two plant species. These transformed plants are being prepared for use in animal studies in collaboration with the Mayo Clinic (Arizona), which have developed a double transgenic mouse model that expresses a human form of the TAA and spontaneously develops mammary gland cancer and lung metastatic lesions. This study is the very first attempt to express tumor-associated antigens in a plant system.

**P11** **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 #1R21A1048374-01

**P10** **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 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.

**P12** **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-1 $\beta$  expression and production of interferon-like cytokines in head-kidney macrophages of rainbow trout (*Oncorhynchus mykiss*). Using a micro-chemotaxis chamber we found that CpGs act as a powerful chemo-attractant in fish *in vitro*. 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*.

# Fifth Annual Conference

## ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

**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 serovar 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 interleukin-2 (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 young chickens, 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 chickens 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.

**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+ cells) 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 when compared to cells from non-vaccinates proliferated ( $p < 0.01$ ) to soluble *M. bovis* antigens. The mean 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.

**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; 6/74 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.

**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 received 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 agglutination 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.

# on Vaccine Research

## ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

**P17**

**WITHDRAWN**

**P19** Role of Langerhans Cells in Epidermal Powder Immunization  
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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 μm) or embedded in the sugar excipient particles (20-50 μm). 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 seronegative, 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.

### PERCENTAGE POSITIVITY OF ANTIBODY RESPONSE

| Day Post infection | Percentage Positivity of Goats immunized with vaccine | Positivity of Goats immunized with adjuvant only | Day Post infection | Percentage Positivity of Goats immunized with vaccine | Positivity of Goats immunized with adjuvant only |
|--------------------|---|--|--------------------|---|--|
| Day 0              | 0%  | 0%   | Day 49             | 100%  | 87.5%  |
| Day 7              | 69%   | 0%   | Day 56             | 100%  | 91.7%  |
| Day 14             | 89.3%   | 0%   | Day 71             | 96.4%   | 70.8%  |
| Day 21             | 96.4%   | 0%   | Day 90             | 100%  | 63%  |
| Day 28             | 96.3%   | 0%   | Day 115            | 82.1%   | 66.7%  |
| Day 35             | 100%  | 0%   | Day 125            | 96.4%   | 33.3%  |
| Day 42             | 96.2%   | 62%  | Day 133            | 92.9%   | 58.3%  |

**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-L52 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 SPf66 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-DRA/DRB1\*0801 molecules indicated that dimorphism does not primarily affect HLA binding. Molecular modelling of HLA-DRA/DRB1\*0801/peptide complexes implies that dimorphic amino acid residues are involved in the interaction with the TCR. Therefore, lack of productive triggering of the TCR by MHC/peptide complexes represents a mechanism of parasite immune evasion associated with antigen dimorphism.

## ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

P21

WITHDRAWN

P22 Evaluation of high molecular weight polysaccharide adhesin from *Staphylococcus aureus*.

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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 b-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 b-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*.

P23

WITHDRAWN

P24

WITHDRAWN

# on Vaccine Research

## ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

### **P25** Parasitic Infection Alters the Quality of the Immune Response to HIV-1 Vaccines

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Over 95% of all HIV-infected people now live in the Developing World. In these countries, chronic parasitic infection adds another level of complexity to vaccine development. Approximately 1.5 billion people carry a parasitic burden. These persons are in a constant state of immune activation with a dominant Th2 type of cytokine profile. Such an immune profile may have an adverse impact on the efficacy of vaccines. We have studied the impact of parasitic infection on the vaccine induced cellular immune response. Balb/c mice were infected with *L. major*. Following the establishment of chronic infection mice were immunized with a DNA HIV-1 vaccine. We observed that the number of CD8 lymphocytes able to IFN-gamma following stimulation with HIV-1 antigen decreased from approximately 200 to 20 CD8 lymphocytes. We observed a slight increase in the number of HIV-1 antigen specific CD4 lymphocytes secreting INF-gamma in the parasitically infected mice as compared to the uninfected and vaccinated mice. Therefore, in subjects chronically infected with parasites it may be beneficial to enhance a vaccine induced cellular response by co-injecting DNA plasmids that code for cytokines that enhance the CD8 immune response. We have observed that the CD8 antigen specific cellular response to the HIV-1 DNA vaccine was significantly enhanced by the addition of IL-15 and indicates that IL-15 could be particularly valuable in developing countries where many individuals have a dominant Th2 cytokine profile caused by chronic parasitic infection.

### **P27** Virus Based Vaccines for Cancer Therapy

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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 NV1020), 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.

### **P26** Pre-Clinical Assay Development for Immunological Screening of a Therapeutic Vaccine Candidate: Evaluation of T-cell Responses to CYP1B1 P450

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Cytochrome P450 proteins are membrane bound drug-metabolizing enzymes in humans. Changes in the expression of P450 are associated with several human diseases. CYP1B1 is an oncoprotein from the P450 family, which is expressed on the surface of several tumors. Induction of cell-mediated responses to this tumor antigen is a critical step in the treatment of disease. Detection of these responses in patients is crucial in demonstrating immunological efficacy of drug candidates. Three strains of mice (C57BL/6, C3H and Balb/c) were immunized and boosted with a microparticle plasmid DNA (pDNA) vaccine encoding the CYP1B1 protein. Synthetic 30mer peptides contained within the CYP1B1 protein sequence were employed for use as test antigens (each peptide was screened for immunogenicity in mice prior to the performance of these experiments). Splens were harvested from the mice and ex vivo IFN-g ELISpot assays were set up using CD3+ and CD4+ enriched T-cell populations. CD3+ and CD4+ T-cell responses were detected to several of the peptides and pools of the peptides. These results demonstrate that 1) the peptides selected for use in these assays can be used to detect T-cell responses in several strains of inbred mice and 2) the therapeutic pDNA vaccine is successful in stimulating cell-mediated immune responses, including CD4+ T-cell responses.

### **P28** HSV Vectors for Cancer and Infectious Disease Immunotherapy

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HSV infects DC efficiently but with minimal replication, suggesting that the lifecycle of HSV usually includes the infection of DC, even though 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 lose 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 have then been further modified by the deletion of ICP4 (replication incompetent vector) or mutation of VP16 (replication competent vector) for ex vivo or direct in vivo use respectively. 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.

# Fifth Annual Conference

## ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

### **P29** Is Crohn's Disease Treatable with Therapeutic Autovaccines?

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**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 Ludwigsburg (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 following the description given in DE 100 21 433-A1. The autovaccines (prepared without further cultivation of micro-organisms) 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 experiments 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

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**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

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Coccidioidin is an important epidemiologic tool that will be utilized for the screening of prospective vaccinees 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

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**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. The proportion 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.

# on Vaccine Research

## ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

### **P33** The Postmarketing Safety Review of Reports of Herpes Zoster After the Administration of VARIVAX® [Varicella Virus Vaccine Live (OKA/MERCK)]

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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®, specimens were requested. 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 from 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 (381/28.1 million doses). This compares to an incidence of 110 HZ cases per 100,000 persons <5 years old with prior varicella infection. (1)

1. Guess H, Broughton DD, Melton LJ, Kurland LT. Population-Based Studies of Varicella Complications. Pediatrics. 1986.

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

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**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; promoting 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.

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

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<sup>1</sup>Epidemiology, University of Michigan, Ann Arbor, MI, <sup>2</sup>Michigan, 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 otitis 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 otitis 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.

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

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#### **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)
- Enrolment 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**

**WITHDRAWN**

**P38** Considerations for Future HAV Vaccine Development

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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.

**P39** 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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**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.

**40**

**WITHDRAWN**

# on Vaccine Research

## ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

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

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**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  $\gamma$  (IFN $\gamma$ ) 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 IFN $\gamma$  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)

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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 ( $\geq 5$  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 ( $\log_2$ ) of  $\geq 6.5$  to RSV/A (OR 3.2 CI 1.4-7.7) and  $\geq 8.0$  to RSV/B (OR 2.8 CI 1.1-6.9) was established against RSV-AH.