VACCINES
Wednesday, July 8

W.77. Oral Immunization with Lactobacillus Casei Vaccine Expressing Human Papillomavirus (HPV) Type 16 E7 Elicits Mucosal Cytotoxic Cellular Immune Response to HPV16 E7

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No therapeutic vaccines against human papillomavirus (HPV)-related intraepithelial neoplasia have succeeded. Previous studies have done intramuscular immunization assessing only systemic cellular immunity to HPV. We here addressed mucosal cellular immunity (MCI) to HPV16 E7 for oral immunization of mice with attenuated Lactobacillus casei expressing HPV16E7 (LacE7).C57/BL6 mice were administered orally five days/week at weeks 1, 2, 4, and 8 with LacE7 or vehicle vaccines. For comparison, GST-fused HPV16 E7 was injected into muscle of the mice once/week at the same weeks. Intestinal mucosal lymphocyte (Integrin α4β7+) or splenocyte were collected at weeks 5 or 9. Induction of E7-specific IFNγ-producing Th1, GranzymeB-producing CD8+ cells, and the cytotoxic activity to E7-positive target cells were addressed.Oral immunization with LacE7 elicited both IFNγ-producing Th1 and GranzymeB-producing CD8+ cells recognizing E7 CTL epitope in the mucosal lymphocyte. Cytotoxic activity to the target cells was also demonstrated. The induction of E7-specific CTL was stronger in the mucosal lymphocyte than splenocyte for oral immunization with LacE7 whereas stronger in splenocyte than the mucosal lymphocyte for intramuscular immunization with GST-E7.Oral immunization with LacE7 elicited E7-specific MCI more effectively than intramuscularly immunization. This vaccine strategy may achieve more effective clinical clearance of HPV-related intraepithelial neoplasia.

W.78. Oral Immunization with Inactivated Non Typeable Haemophilus Influenzae (NTHi) Reduces the Severity of Acute Exacerbations of Chronic Obstructive Pulmonary Disease (COPD)

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We postulate that an oral immunisation strategy in COPD, inducing a selective and specific T cell response (previous study), can protect against acute exacerbations. 73 subjects were studied for 6 months in a placebo-controlled Phase 2 trial following oral NTHi (HI-164OV). Results: (see table) Culture positive sputa halved following HI-164OV ($p<0.05$). No significant change in IgA antibody in serum or secretions. Conclusion: These results support: 1) the thesis that gut-derived T cells control mucosal inflammation, 2) benefit for oral HI-164OV immunisation in COPD (a major unmet medical need); 3) the importance of bacterial colonisation in the pathogenesis of COPD exacerbations.

	Total COPD			Moderate-Severe COPD*		
	HI-164OV (36)	Placebo (37)	Protection % (p-value)	HI-164OV (18)	Placebo (20)	Protection % (p-value)
Acute episodes (per subject)						
• Total	1.0	1.35	26 (0.16)	1.22	1.45	16 (0.55)
• Antibiotic-treated	0.78	1.03	24 (0.26)	0.83	1.15	28 (0.33)
• Corticosteroid-treated	0.28	0.57	51 (0.06)	0.28	0.75	63 (0.05)
• Hospital-treated	0.138	0.351	60 (0.08)	0.055	0.55	90 (0.04)
Recurrent episodes	0.22	0.49	55 (0.03)	0.33	0.55	40 (0.55)
Antibiotic therapy (per subject) total days	7.89	19.86	60 (0.01)	7.56	27.15	72 (0.01)
Duration mean days	14.7	17.3	15 (0.19)	14.3	22.7	37 (0.01)
	*FEV_1<50% predicted normal					

W.79. Intranasal HIV DNA Vaccination in Neonatal Mice Induces Gut Humoral and Cellular Immunity Simultaneously with Treg Generation

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Mucosae in the vagina and gastrointestinal tract are important primary infection sites for HIV. This study evaluated the effect of intranasal (IN) administration in neonatal mice of a chimeric DNA vaccine encoding HIV-GAG and LAMP protein, which directs the traffic of the protein to the MHC II compartments and only HIV-GAG vaccine. Seven day-old BALB/c mice were immunized with either Lamp/gag or gag vaccines by IN and intradermal (ID) route and boosted twice. The IN immunization with 20μg of the Lamp/gag stimulated synthesis of anti-GAG IgA and IgG in gut mucosa, with increased total IgG synthesis, and anti-GAG IgG in serum. Similar profile of splenic and gut CD8 response by Lamp/gag and gag mice was verified by the frequency of IFN-γ spot-forming cells (SFC) to GAG peptides, percentage of CD8+/pentamer+ cells, *in vivo* CTL response and secretion of cytokines such as TNF-α and IFN-γ. As for the CD4 response, Lamp/gag mice recognized twice the number of pools than gag mice with increased frequency of IFN-γ SFC and IL-4 SFC to GAG peptides. After *in vitro* stimuli with GAG class II peptide, gag mice splenic cells produced increased levels of TNF-α and IL-10, whereas Lamp/gag mice secreted higher levels of IFN-γ and IL-2. Mesenteric lymph nodes (MLN) from Lamp/gag and gag mice showed augmented percentage of CD4+CD25+FOXP3+ Treg cells, in accordance to the increased levels of TGF-β1 observed in intestinal washes. Dendritic cells (CD11c+CD8α+) from MLN of Lamp/gag mice had their expression of I-Ad molecules increased. Immunization with gag or Lamp/gag by was immunogenic in the neonatal period and capable of inducing regulatory mechanisms, although only Lamp/gag vaccine induced humoral and cellular immune response and increased activation of MLN DCs. Supported by: Brazilian Ministry of Health, FAPESP and LIM-56/HCFMUSP.

W.80. Evaluation of a Non-ionic Surfactant (Span 60) as an Oral Vaccine Adjuvant

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Given the increasing field records of Newcastle disease virus (NDV) vaccine failures, there is a need for newer vaccines and vaccine adjuvants. In the present study, we evaluated the effect of a non-ionic surfactant (Span 60) on the immunogenicity of an NDV antigen in chickens. The LaSota strain of NDV antigen was formulated in a niosomal base (Group 1) and compared with the commercial vaccine (positive control)(Group 2). The negative control contained no vaccine. The above treatments were administered to thirty specific pathogen free birds in three treatment groups. The primary dose was administer at 3 weeks and booster dose at 5 weeks. Statistical analysis (using Genstat release 72DE rc/windows) showed 460% and 580% increment in HI titre of Group 1 and Group 2 respectively after first post vaccination assay and 640 % and 600% overall increment in HI titre after second post vaccination assay in Group 1 and Group 2 respectively. In conclusion, the niosomal based vaccine had a higher and prolonged immune response.

W.81. Progress Toward Developing a Live Recombinant Attenuated Salmonella Vaccine to Induce Cross-protective Immunity to Enteric Bacterial Pathogens

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We genetically engineered serovar Typhimurium, Paratyphi A and Typhi vaccines to display regulated delayed attenuation and synthesis of protective antigens specified by codon-optimized DNA sequences from multiple enteric bacterial pathogens. Vaccines are grown to enable display of wild-type capabilities to survive host defense stresses and colonize effector lymphoid tissues before manifesting *in vivo* attenuation to preclude disease symptoms and synthesize protective antigens to induce immune responses. We engineered strains to eliminate or decrease synthesis of serotype-specific antigens, expose or over express immunologically cross-reactive surface antigens, to diminish induction of gastroenteritis while retaining abilities to recruit innate immunity, and exhibit biological containment to preclude persistence *in vivo* or survival if excreted. Strains are totally safe at high doses to newborn and pregnant mice. We are developing vaccines that by over-expression of cross-reactive surface antigens and delivery of pathogen-specific conserved protective antigens diminish colonization and/or infection of chickens and mice by diverse *S. enterica* serotypes, *Escherichia coli Clostridium perfringens*, *Shigella* species/serotypes, *Campylobacter jejuni* and *Yersinia enterocolitica* and pseudotuberculosis. We are continuing evaluations of these vaccines, are adding expression of additional protective antigens against enteric pathogens using multiple balanced-lethal plasmid vector systems and are initiating efforts with others to prevent infections by parasite enteric pathogens. These antibiotic-sensitive vaccines are designed for oral needle-free delivery to newborns and infants. This research was supported by the Bill and Melinda Gates Foundation, Ellison Medical Foundation, NIH and USDA.

W.83. Intestinal Helminth Infection Compromises Oral and Parenteral Vaccine Efficacy

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The impact of endemic parasitic infection on vaccine efficacy is an important factor for vaccine development that is little explored. Epidemiological studies and mouse models of allergy and inflammation suggest that helminth infection may promote immune suppression by inducing regulatory cells that modulate immune responses. We examined whether intestinal infection with the nematode Heligmosomoides polygyrus altered antigen-specific humoral responses to intramuscular and oral vaccination. We found that chronic intestinal helminth infection significantly reduced both Th2-skewed antibody responses to intramuscular vaccination with ovalbumin (OVA) adsorbed to alum and Th1-skewed antibody responses to oral vaccination with Salmonella-OVA, a clinically relevant recombinant Salmonella vaccine. Impaired antibody responses in helminth-infected, orally vaccinated mice were not due to an inability to respond to vaccine antigens since activated antigen-specific CD4+ T cells accumulated in draining mesenteric lymph nodes (MLNs) of helminth-free and helminth-infected mice. Helminth infection also increased the frequencies of adoptively transferred antigen-specific CD4+ T effector cells producing IFN-γ and IL-4, and importantly, antigen-specific CD4+ IL-10-producing T cells in the MLN. These findings suggest that IL-10-secreting CD4+ T regulatory cells may suppress vaccine-induced humoral responses in helminth-infected individuals and underscore the potential need to treat parasitic infection before mass vaccination in helminth-endemic areas.

W.84. Adjuvant Mechanisms of Recombinant Antigen-enterotoxin A2/B Chimeric Mucosal Immunogens

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Previous studies have demonstrated that recombinant chimeric proteins consisting of the saliva-binding region (SBR) of Streptococcus mutans AgI/II coupled to the A2/B subunits of cholera toxin (CT) or type II *E. coli* labile toxins LTIIa and LTIIb induce strong antibody responses against AgI/II. This study investigated the adjuvant mechanisms and differences between chimeric SBR-CTA2/B,

SBR-LTIIaA2/B, SBR-LTIIbA2/B proteins in comparison with SBR and with CT as a positive control. Mice were immunized intragastrically (i.g.), and antigen presenting cells (APC) from Peyer's patches (PP), mesenteric lymph nodes (MLN), and spleen were assayed by flow cytometry for the expression of surface markers and costimulatory molecules. All chimeric proteins as well as SBR greatly increased the expression of costimulatory molecules on splenic APC, and of MHC-II on splenic dendritic cells (DC) and macrophages, to a greater extent than CT. All the proteins affected the number of DC in PP and the expression of costimulatory molecules. All chimeric proteins and SBR increased the number of PP macrophages but decreased the expression of CD40 and CD86 on B cells. The pattern of responses by APC in different tissues suggested their migration from PP to MLN and spleen.

W.85. Pneumococcal Surface Protein A-specific S-IgA Abs Prevent Nasal Colonization of *Streptococcus Pneumoniae*

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This study was designed to investigate whether mucosal IgA antibodies (Abs) induced by a pneumococcal surface protein A (PspA)-based nasal vaccine play a key role in the prevention of streptococcal colonization. IgA knockout (IgA-/-) and haplotype control mice were nasally immunized three times at weekly intervals with PspA plus flt3 ligand plasmid (pFL). One week after the last immunization, mice were challenged with the EF3030 strain of *Streptococcus pneumoniae* via the nasal route. Prior to challenge, PspA-specific S-IgA and IgG Ab responses were determined. Five days after challenge, nasal washes (NWs) and nasal passages (NPs) were collected and the numbers of bacterial colonies were determined. Mice immunized with PspA plus pFL had significantly higher levels of PspA-specific S-IgA and IgG Ab responses in plasma and NWs when compared with naïve mice. Although IgA-/- mice given nasal PspA plus pFL had significantly high levels of PspA-specific IgG Abs, high numbers of colony-forming units were detected in NWs and NPs. In contrast, vaccinated WT mice showed essentially no bacteria in the nasal cavity. These results show that PspA-specific S-IgA Abs play an important role in the prevention of *S. pneumoniae* colonization in the nasal cavity. Supported by NIH grants AG025873 and DE12242.

W.86. Sublingual Immunization with Phosphorylcholine has a Potential of Broad Spectrum Vaccine Against Upper Airway Infections

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Phosphorylcholine (PC) is recognized as a structural component of a wide variety of Gram-positive and -negative bacterium. The entry of *S. pneumoniae* into human endothelial cells is dependent upon the interaction between PC expressed on bacterial surface and PAF receptor of host epithelium. The findings suggest that the expression of PC on bacterial surfaces is associated with bacterial invasion to upper airway. Thus PC-containing protein is a potential broad spectrum vaccine antigen that might be used to prevent upper airway infections caused by *S. pneumoniae* and *H. influenzae*. Previously we demonstrated that PC specific immune responses at the mucosal site as well as the systemic site were induced by sublingual immunization with PC-KLH and CT as well as intranasal immunization in mice. In the present study, saliva and nasal wash samples were obtained after sublingual immunization, and the cross-reactivity of PC-specific secretory IgA against a wide variety of *S. pneumoniae* and *H. influenzae* strains was examined. The results suggested that sublingual immunization with PC has a potential of broad spectrum vaccine against upper airway infections.

W.87. Human Monoclonal Antibodies that Inhibit Mutans Streptococcal Glucosyltransferase Activity and Biofilm Formation

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Active immunization with *S. mutans* GTF or passive administration of polyclonal antibody to GTF has been associated with reductions in experimental dental caries. Thus timely exposure of the human oral cavity to antibody of this specificity should block entry of mutans streptococci into the developing biofilm. Since passive vaccine approaches using human monoclonal antibody may be safest, we prepared human IgG1 monoclonal antibody which had the ability to inhibit *S. mutans* GTF function and biofilm formation. Human phage display libraries (2.7 X 1010 members from B cells of 57 donors) were screened against GTF-coated surfaces to select human single-chain variable region fragments (scFvs) against GTF. Recombinant human monoclonal antibodies (IgG1) were then prepared and GTF-binding activities of these pure antibodies were confirmed by ELISA. Two of these antibodies significantly inhibited the ability of *S. mutans* GTF to synthesize glucan from sucrose. They also significantly ($p<0.03$) reduced *S. mutans* biofilm formation in an *in vitro* plate assay. These human monoclonal antibodies interfere with important aspects of cariogenic *S. mutans* accumulation, thus have potential for passive antibody application to children. Support: DE-04733 from NIDCR.

W.88. Oral Vaccination with MucoRice™-CTB Provides Long-term Protective Immunity against Live *Vibrio Cholerae*- and *Enterotoxigenic Escherichia Coli*-induced Diarrhea

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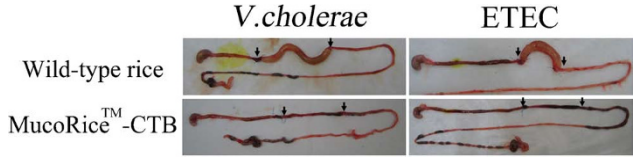
We previously showed that oral vaccination with MucoRice™-CTB induced cholera toxin B subunit(CTB)-specific immune responses with protective immunity in both systemic and mucosal compartments. In order to further advance the MucoRice™-CTB development for application in human, we further addressed the protective efficiency against live *V. cholerae*. As results, oral vaccination with MucoRice™-CTB provides long-term protective immunity capable of inhibiting the onset of diarrhea caused by live *V. cholerae*-induced diarrhea for at least 6 months. In addition, because CTB-specific secretory IgA (SIgA) induced by oral vaccination with MucoRice™-CTB also cross-reacted with *E. coli* heat labile toxin B subunit, MucoRice™-CTB immunized mice protected against live enterotoxigenic *E. coli*-induced diarrhea. Taken together, these results indicate that MucoRice™-CTB-induced SIgA would be key element for inhibition of diarrhea caused by *Vibrio cholerae*- and enterotoxigenic *Escherichia coli*.



W.90. Nasal Influenza Vaccine Combined with CpG-ODN and pFL as Mucosal Adjuvant Induces Two Subsets of Dendritic Cells for Th1-and Th2-type Cytokine Responses

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Our previous study demonstrated that nasal influenza PR8-Ag plus a combination of CpG ODN and plasmid expressing Flt3 ligand (pFL) induced Ag-specific secretory (S)-IgA antibody (Ab) responses and protective immunity against influenza challenge. To understand the cellular and molecular mechanisms for the induction of influenza-specific immunity by a combined nasal adjuvant, we characterize Th1- and Th2-type cytokine and dendritic cell (DC) responses in mice given PR8-Ag (0.2 μg) plus pFL (50 μg) and CpG (10 μg). One week after the last immunization, lymphocytes from NALT, posterior cervical lymph nodes (pCLN), and nasal passages (NPs) were collected and analyzed for their phenotypes and intracellular cytokine production by flow cytometry. Significant increased numbers of both IFN-γ and IL-4 producing cells were noted in vaccinated mice when compared with controls. Remarkably high numbers of plasmacytoid DCs and myeloid-type DCs were induced after nasal immunization in all tissues. These results suggest that two distinct subsets of DCs are the key players for the induction of balanced Th1- and Th2-type cytokine responses and following influenza-specific protective immunity. We are currently investigating the cytokine responses and APC function by DC subsets in the NALT and NPs. Supported by NIH grants AG025873 and DE12242.

W.92. Inactivated Whole-cell Vaccine Delivered Intranasally Protects Mice Against Pneumonic Plague

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Plague is the deadliest manifestation of infectious disease that has killed over 200 million people worldwide. Formalin-inactivated *Yersina pestis* Plague vaccine USP was used for plague prevention. This vaccine was given by intramuscular route. However, the license for the Plague vaccine USP has been revoked due to lack of efficacy against pneumonic plague, and reactogenicity. We have recently found that intranasal administration of paraformaldehyde-inactivated *Y. pestis* CO92 organisms were immunogenic and efficacious in protection against pneumonic plague. Mice were immunized with 10^8 CFU of inactivated *Y. pestis* with or without 1μg IL-12 on days 0 and 21. Immunized mice were challenged on day 60 with lethal *Y. pestis* CO92. The immune mice generated robust antibody responses and showed 100% survival from lethal challenge. This inactivated vaccine also induced BALT-like structures in the lung that corresponded with recruitment of B lymphocytes, T lymphocytes, and MHC-II+ APCs in to the lung parenchyma. Further studies are underway to optimize the vaccine dose for mucosal delivery with IL-12 and characterize the protective mechanisms. Acknowledgement: The authors acknowledge financial support from the DOD-ONR (Award no. N00014-06-1-1176).

W.93. Enteric Adenovirus 41 Vector Enhances Induction of Intestinal Cellular Immunity by Oral Priming

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Human immunodeficiency virus-1 (HIV-1) infection is characterized by rapid onset of intestinal T cell depletion that initiates the progression to AIDS. The induction of protective immunity in the intestinal mucosa therefore represents a potentially desirable feature of a preventive AIDS vaccine. In this study, we have evaluated the ability of an enteric adenovirus, recombinant adenovirus (rAd) 41, to elicit intestinal and systemic

immune responses by different immunization routes, alone or in combination with rAd5. rAd41 expressing HIV envelope (Env) protein induced cellular immune responses comparable to rAd5-based vectors after either a single intramuscular injection or a DNA prime/rAd boost. Oral priming with rAd41-Env followed by intramuscular boosting with rAd5-Env stimulated a more potent CD8+ T cell response in the small intestine than the other immunization regimens. Furthermore, direct injection of rAd41-Env into ileum together with intramuscular rAd5-Env boosting increased Env-specific cellular immunity markedly in mucosal as well as systemic compartments. These data demonstrate that heterologous rAd41 oral or ileal priming with rAd5 intramuscular boosting elicits enhanced intestinal mucosal cellular immunity and that oral or ileal vector delivery for primary immunization facilitates the generation of mucosal immunity.

W.94. Intramuscular Delivery of a Cholera DNA Vaccine Primes both Systemic and Mucosal Protective Antibody Responses Against Cholera

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Cholera is a potentially lethal diarrhea disease caused by the gram-negative bacterium Vibrio cholera. The need for an effective cholera vaccine is clearly indicated but the challenges of eliciting both systemic and mucosal immune responses remains a significant challenge. In the current report, we discovered that a DNA vaccine expressing a protective cholera antigen, cholera toxin B subunit (CTB), delivered parenterally can elicit both systemic and mucosal anti-CTB antibody responses in mice. The priming effect by DNA immunization was demonstrated by higher mucosal antibody responses following one boost with the inactivated cholera vaccine (KWC-B) delivered by orally when compared to the twice oral administration of KWC-B alone. This finding indicates that DNA vaccines delivered parenterally are effective in eliciting mucosal protective immune responses - a unique advantage for DNA vaccination that has not yet been well realized and should bring value to the development of novel vaccination approaches against mucosally transmitted diseases.

W.96. Enhanced Ova Presentation After Nasal Application of OVA Containing Nano-particles

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To enhance efficacy of mucosal vaccines, encapsulation of antigen into nano-particles is a promising approach. Here, we show that OVA encapsulated in poly(lactic-co-glycolic acid) tripolyphosphate (PLGA) or in PLGA- N-Trimethyl chitosan (TMC) particles is more efficiently presented to CD4+ DO11.10 T cells compared to soluble OVA. *In vitro* 25 ng/ml OVA encapsulated in PLGA or PLGA-TMC particles adequately induced proliferation in 85,5% (±7,6) and 87,9% (±2,4) of T cells after 72 hours, respectively. Whereas only 22,7% (±7,0) of T cells divided when soluble OVA was added to the culture. *In vivo*, T cell activation was addressed by adoptive transfer of CFSE-labeled CD4+ DO11.10 T-cells to naive Balb/c mice. Three days after nasal application of 20 μg OVA PLGA-TMC-OVA particles induced visible T cell proliferation in cervical lymph nodes. However, *in vitro* re-stimulation of CLN cells with soluble OVA protein showed that both PLGA and PLGA-TMC particles had primed the immune response *in vivo*. Most antigen specific T cells isolated from PLGA-TMC or PLGA particle treated mice (84% and 75%, respectively) compared to 50% in the Ova treated group. It is clear from our data that PLGA(TMC)-OVA nanoparticles enhance the immunogenicity of OVA protein both *in vitro* and *in vivo* by enhancing T cell activation.

W.97. Adjuvant Combinations: A Way to Achieve More Potent Immune Responses

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The development of new vaccines represents a challenge. Many vaccines need adjuvants to boost their effectiveness, and one possible way to achieve more efficient vaccine formulations with minimal side effects is to combine different adjuvants. The aim of this study is to optimize both cellular and humoral immune responses using adjuvant combinations. Two new generation adjuvants were chosen. CTA1-DD is based on the enzymatically active A1 subunit of cholera toxin (CT) and functions through the activation of G proteins. CTB-CpG links the B subunit of CT and CpG oligonucleotide, and stimulates the immune system through TLR9. These were tested for their ability to synergize with each other and an array of pathway-specific adjuvant molecules in the induction of immune responses. Mice were intranasally immunized with ovalbumin (OVA) coupled to nitrophenyl (NP) together with the adjuvants. Immune responses were subsequently measured as proliferation of splenocytes following OVA stimulation and the presence of NP-specific antibodies in serum. The identification of combination adjuvants with increased efficiency aims to provide improved vaccine formulations, and will contribute to a better understanding of the mode of action of different adjuvants.

W.98. Systemic Antibody Titers at Mucosal Surfaces and Cellular Immunogenicity Induced by Bacteriophage T4 Displaying HIV-1 Envelope Gp41 Proteins Complexed with Liposomes Containing Lipid A

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Since the main route of transmission of HIV-1 is via heterosexual contact across mucosal surfaces, any HIV-1 vaccine developed needs to generate protective mucosal responses. In an attempt to induce robust systemic antibody titers at mucosal surfaces and cellular immune responses, HIV-1 gp41 cytoplasmic outer domain (amino acid 724-745) and trimerized C- heptad repeat (aa 628-661) containing a portion of MPER (aa 662-673) displayed on separate bacteriophage T4 capsids were used as antigens in conjunction with full length gp41 in the presence or absence of liposomes containing glucosyl ceramide and lipid A. BALB/c mice were immunized by the intramuscular route at 0, 3, and 6 weeks. Both vaccine formulations induced gp41-specific IgG and IgA antibodies (titers of 100 -1000) in vaginal washes at week 7, as well as, serum IgG antibodies (titers of 10^6). Systemically, TNF-alpha and IFN-gamma were induced as measured by cytokine bead array. Antigen-specific ELISPOT and T cell proliferation were also obtained. Phage T4-liposome is a novel multi-component HIV-1 protein-lipid platform that generates systemic antibodies at mucosal surfaces and cellular immunity. The views expressed are those of the authors and should not be construed to represent the positions of the Department of the Army or Department of Defense.

W.101. Synergistic Induction of Antigen-specific Immune Response by M Cell Targeting Ligand-mediated Mucosal Vaccination

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Mucosal immune responses are the most efficient defense system to protect body against the infectious agents and the responses could be induced efficiently through mucosal immunization. Consequently, M cell targeting is believed important for successful mucosal immunization since antigen delivery across the mucosal surface is prerequisite for stimulation of mucosal immune system. In this study, to search for reliable peptide ligands which can act as mucosal adjuvants, we have selected the peptides which can bind onto the cultured M cell model using Caco-2 and Raji co-culture system by screening the phage display library. Interestingly, one of these peptides showed high homology to outer membrane protein of Yersinia enterocolitica-like strains which are well-known pathogen capable of directly invading and destroying M cells. A fusion protein with EGFP or VP1 antigen of foot-and-mouth disease virus and a peptide was efficiently delivered into the Peyer's and induced the antigen specific Th2 type immune response after oral administration. In addition, when we applied an antimicrobial peptide onto the M cell system, the peptide modulated the M cell environment. Therefore, appropriate peptides could be used as adjuvant or modulator for mucosal immune response induction.

W.102. Rotavirus-like Particle (VLP) Vaccines with/without Attenuated Rotavirus Priming Induce Heterotypic Rotavirus Antibody Responses but Low Protection Rates to Heterotypic Rotavirus Challenge

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Nonreplicating VLP vaccines against human rotavirus (HRV) pose fewer safety concerns compared to live rotavirus vaccines but whether they induce heterotypic protection is unclear. Gnotobiotic piglets were vaccinated orally/intranasally with attenuated AttMHRV+2/6VLP-ISCOM or 2/6/7VLP+2/6/4VLP-ISCOM vaccines against G3P1A HRV (M Strain) or mock (controls), then were challenged with heterotypic G1P1A virulent WaHRV (Wa strain). We evaluated numbers of HRV-specific antibody-secreting cells (ASCs) and virus neutralizing (VN) antibody titers against Wa HRV. The highest numbers of HRV-specific IgA and IgG ASCs pre-challenge were induced in ileum by the AttMHRV+2/6VLP vaccine, whereas the 2/6/7VLP+2/6/4VLP vaccine induced IgA and IgG ASCs in ileum only post-WaHRV challenge. Both vaccines induced statistically similar levels of serum IgG antibodies pre-challenge, but the AttMHRV+2/6VLP vaccine stimulated significantly higher serum IgA pre-challenge and serum IgG and IgA antibodies post-challenge. VN titers to the Wa strain were statistically higher pre-challenge for the AttMHRV+2/6VLP vaccine than the 2/6/7VLP+2/6/4VLP vaccine but statistically similar post-challenge. Both vaccines induced similar low levels of heterotypic protection against diarrhea (36-37%) compared to 71% for the homotypic G1 AttWaHRV+2/6VLP vaccine. Thus both candidate vaccines (replicating and non-replicating, G3) induced cross-reactive intestinal and systemic antibodies and similar but low protection rates against a heterotypic G1 WaHRV challenge.

W.103. The F4 Receptor, a New Target to Induce Intestinal Immunity Against Enteropathogens

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Vaccination against intestinal infections remains a tremendous challenge. Very few antigens can induce an intestinal immune response when given orally. We have proven that in pigs purified F4 fimbriae of enterotoxigenic *E. coli* (ETEC) can be added to the list of rare soluble oral immunogens, since oral administration of F4 to F4 receptor positive pigs induces an intestinal immune response providing complete protection against challenge infection. Most if not all of the soluble antigens that are

immunogenic via the oral route bind to receptors on enterocytes and are polymeric in nature. Also F4 is a polymer that binds to glycoproteins and glycolipids. We demonstrated that F4 binds to aminopeptidase N and that this binding results in endocytosis of F4. Transcytosis was demonstrated using intestinal loops en an epithelial cell line. Oral administration of F18 fimbriae does not lead to a mucosal immune response. Therefore, the adhesin Fed F of F18 was targeted to aminopeptidase N by conjugating it to F4. This conjugation induced a local FedF-specific immune response which led to a reduction in excretion after challenge infection with F18+*E. coli* . This is a first step towards the development of a combined.