Infections – Intestines
Tuesday, July 7

T.73. Neonatal Sublingual Administrated with Sonicated Salmonella Antigens and Mucosal Adjuvants Protect Mice Against Enteric Pathogens

Ching-Feng Huang[1], Ho-Jen Peng[2]
[1]Tri-Service General Hospital, Taipei, Taiwan; [2]Taipei Veterans General Hospital, Taipei, Taiwan

Background: Newborns are at risk for many infectious diseases. Induction of systemic and mucosal immune responses in a simple, non-invasive pathway is important to protect newborn against mucosal pathogens. Methods: Neonatal BALB/c mice received daily sublingual vaccination with sonicated Salmonella proteins (SSP) alone, or plus adjuvant CpG or cholera toxin (CT) in the first five days of life. Seven weeks later, they were boosted. Control mice were similarly treated with PBS only. Results: Saliva specific secretory IgA antibody responses were significantly increased after neonatal sublingual administration with SSP plus CpG or CT. Vaccination with SSP and CpG enhanced serum specific IgG2a antibody responses and IFN-γ production in both cervical lymph node (CLN) and spleen cells. On the contrary, vaccination with SSP and CT increased IL-4, IL-5, IL-6 and IFN-γ production in both CLN and spleen cells and serum specific IgG1 and IgG2a antibody responses. After oral challenge with live *S. enteritidis*, those neonatally vaccinated with SSP and CT or CpG maintained oral intake and protected from intestinal necrosis and mortality. Indeed, the vaccinated mice with higher specific IgG and SIgA antibody levels had a better survival rate. Conclusion: Neonatal sublingual vaccines may play a crucial role for the protection against enteric pathogens.

T.74. Innate Immunity Mediated by MyD88 Signal is not Essential for Induction of LPS-specific B Cell Responses but is Indispensable for Protection Against *Salmonella Enterica* Serovar Typhymurium Infection

Hyung-Jun Yang[1], Hyun-Jeong Ko[1], Jin-Young Yang[1], Doo-Hee Shim[1], Roy Curtiss[2], Mi-Na Kweon[1]
[1]International Vaccine Institute, Seoul, Korea, South; [2]The Biodesign Institute, Arizona State University, Tempe, AZ

Salmonella organisms are gram-negative and facultative anaerobic bacteria that cause typhoid fever in humans. In this study, we evaluated LPS-specific adaptive immunity in innate immune-deficient mice after oral administration of attenuated *S. Typhimurium* strains. Of interest, identical levels of LPS-specific IgG and IgA antibodies were elicited in the systemic (i.e., serum and spleen) and mucosal (i.e., fecal extract and small intestine) compartments of wild-type, TLR4-/-, and MyD88-/- mice following oral vaccination with recombinant attenuated *S. Typhimurium* (RASV). Depletion of CD4+ T cells during RASV vaccination completely abrogated the generation of LPS-specific antibodies in MyD88-/- mice. In addition, mRNA expression levels of a B-cell activating factor of the TNF family (BAFF) were significantly increased in the spleens of MyD88-/- mice after oral administration, implying that T cell-independent B cell switching might be also enhanced in the MyD88 signal-deficient condition. Of most interest, orally vaccinated MyD88-/- mice that possessed high levels of LPS-specific IgG and IgA, which had neutralizing effect against Salmonella, died earlier than non-vaccinated wild-type mice following lethal oral challenge with virulent Salmonella species. These results suggest that innate immunity mediated by MyD88 signal is dispensable for induction of LPS-specific antibody responses following oral administration of attenuated Salmonella strains but indispensable for efficient protection.

Innate immunity mediated by MyD88 signal is not essential for induction of LPS-specific B cell responses but is indispensable for protection against *Salmonella enterica serovar Typhymurium* infection

Hyung-Jun Yang*, Hyun-Jeong Ko*, Jin-Young Yang*, Doo-Hee Shim*, Roy Curtiss III†, and Mi-Na Kweon*

**Mucosal Immunology Section, Laboratory Science Division, International Vaccine Institute, Seoul, Korea 151-818; † Center for Infectious Diseases and Vaccinology, The Biodesign Institute, Arizona State University, Tempe, AZ 85287-5401, USA*

Salmonella organisms are gram-negative and facultative anaerobic bacteria that cause typhoid fever in humans. In this study, we evaluated LPS-specific adaptive immunity in innate immune-deficient mice after oral administration of attenuated *S. Typhimurium* strains. Of interest, identical levels of LPS-specific IgG and IgA antibodies were elicited in the systemic (i.e., serum and spleen) and mucosal (i.e., fecal extract and small intestine) compartments of wild-type, TLR4-/-, and MyD88-/- mice following oral vaccination with recombinant attenuated *S. Typhimurium* (RASV). Depletion of CD4+T cells during RASV vaccination completely abrogated the generation of LPS-specific antibodies in MyD88-/- mice. In addition, mRNA expression levels of a B-cell activating factor of the TNF family (BAFF) were significantly increased in the spleens of MyD88-/- mice after oral administration, implying that T cell–independent B cell switching might be also enhanced in the MyD88 signal-deficient condition. Of most interest, orally vaccinated MyD88-/- mice that possessed high levels of LPS-specific IgG and IgA, which had neutralizing effect against *Salmonella*, died earlier than non-vaccinated wild-type mice following lethal oral challenge with virulent *Salmonella* species. These results suggest that innate immunity mediated by MyD88 signal is dispensable for induction of LPS-specific antibody responses following oral administration of attenuated *Salmonella* strains but indispensable for efficient protection.

*This work was supported by the Bill & Melinda Gates Foundation (No. 37863), NIH R01-AI056289 and a Korea Science and Engineering Foundation (KOSEF) grant funded by the Korean government (MEST) (No. 2007-04213 and R01-2008-000-10649-0)

T.75. Histopathology and Cytokine Responses Following Infection of *Shigella Sonnei* Strains in Gnotobiotic Piglets

Kwang-il Jeong[1], Shoshana Barnoy[2], Malabi Venkatesan[2], Saul Tzipori[1]
[1]Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA; [2]Walter Reed and Army Institute of Research (WRAIR), Silver Spring, MD

An animal model for shigellosis in humans is essential to study the pathogenesis of the disease and provide a tool to develop and evaluate vaccine candidates against the disease. Here, we describe a gnotobiotic (GB) pig model for shigellosis caused by Shigella sonnei wild type (Mosley). We have used this model to assess the occurrence, if any, of side-effects induced by *S. sonnei* vaccine candidate (WRSs3) and a mutant strain (ShET2) constructed at WRAIR. Piglets aged 2–3 days were inoculated orally with either Mosley, WRSs3 strain lacking the virulence plasmid

VirG(IcsA), enterotoxin genes and lipid A acyltransferase gene, or ShET2 mutant strain lacking enterotoxin genes. The Mosley induced moderate to severe diarrhea 2~4 days post inoculation (PI), with severe mucosal damage mainly to the cecum and colon in piglets 1~3 days PI. The WRSs3, in contrast, caused minimal clinical symptoms or histopathological lesions, while the ShET2 mutant induced moderate symptoms and lesions. Cytokine analysis in rectal swabs revealed markedly elevated pro-inflammatory cytokines (IL-8 > IL-1 >IL-6) by Mosley 1~5 days PI, and 1~3 days PI by ShET2 mutant. In contrast, WRSs3 elicited significantly lower levels of these cytokines, but comparable amounts of IL-10 4~8 days PI. The current results indicate GB piglets develop the disease features of shigellosis similar to those observed in humans, and this model was able to discriminate between a fully virulent strain and vaccine candidate, suggesting GB piglet as an animal model in which to evaluate vaccine candidates and therapeutic agents against shigellosis. This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. N01-AI-30050.

T.76. A Variant in Long Palate, Lung and Nasal Epithelium Clone 1 is Associated with Cholera in a Bangladeshi Population

Regina LaRocque[1], Pardis Sabeti[2], Priya Duggal[3], Fahima Chowdhury[4], Ashraful Khan[4], Lauren Lebrun[1], Jason Harris[1], Edward Ryan[1], Firdausi Qadri[4], Stephen Calderwood[1]
[1]Massachusetts General Hospital, Boston, MA; [2]Massachusetts Institute of Technology, Cambridge, MA; [3]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD; [4]International Centre For Diarrhoeal Disease Research, Dhaka, Bangladesh

Cholera is the prototypical mucosal infection. Infection with the causative organism, *Vibrio cholerae*, leads to a dehydrating diarrheal illness that can be rapidly fatal in the absence of specific treatment. Cholera is an historic scourge and, like similar infectious diseases, may have influenced the evolution of the human genome. We report here the results of the first candidate gene association study of cholera. In a family-based study of 76 pedigrees from Dhaka, Bangladesh, we evaluated the association between cholera and five candidate genes – the cystic fibrosis transmembrane receptor; lactoferrin; long palate, lung and nasal epithelium clone 1 (LPLUNC1); estrogen related receptor alpha; and calcium activated chloride channel 1. We found a significant association with a marker in the promoter region of LPLUNC1, a member of a family of evolutionarily conserved innate immunity proteins. A previous microarray-based study of duodenal biopsies revealed significantly increased expression of LPLUNC1 in cholera patients compared to healthy control subjects. Our results suggest that variation in host innate immune responses may influence the outcome of exposure to *V. cholerae* in an endemic setting.

T.77. Transmissibility of an R5 Clade C SHIV Across Different Mucosae in Rhesus Macaques Parallels the Relative Risks of Sexual HIV-1 Transmission via Different Routes

Nagadenahalli Siddappa[1], Agnes Chenine[1], Victor Kramer[1], Gaia Sciaranghella[1], Robert Rasmussen[1], Micheal Santosuosso[2], Mark Poznansky[2], Rama Amara[3], Christopher Souder[3], Daniel Anderson[3], James Else[3], Frank Novembre[3], Elizabeth Strobert[3], William Secor[4], Ruth Ruprecht[1]
[1]Dana Farber Cancer Institute, Boston, MA; [2]Massachusetts General Hospital, Boston, MA; [3]Emory University, Atlanta, GA, GA; [4]Centers for Disease Control and Prevention, Atlanta, GA, GA

An estimated 90% of HIV transmissions worldwide occur mucosally; most involve R5 strains. In humans, the risks of sexual HIV acquisition are rectal>vaginal>oral; whether this rank-order is impacted by mucosal lacerations is unknown. To assess relative virus transmissibility, we used an identical stock of SHIV-1157ipd3N4, a simian-human immunodeficiency virus encoding a primary HIV clade C env (SHIV-C), to perform challenges across intact mucosae in rhesus macaques (RM). We found the same rank-order as that reported in humans, and relative RM mucosal penetrability fell within the relative risk estimates obtained in epidemiological studies. To test whether inflammation facilitates virus transmission, we established a RM model of localized buccal inflammation. Systemic infection only occurred across inflamed but not normal buccal mucosa. Our primate data recapitulate virus transmission risks observed in humans, thus establishing R5 SHIV-C in RM as model system to study cofactors involved in human mucosal HIV transmission and its prevention.

T.78. *Vibrio Cholerae* in Patients Co-infected with Intestinal Parasites in Bangladesh

Jason Harris[1], Michael Podolsky[1], Bhuiyan Taufiqur[2], Fahima Chowdhury[2], Ashraful Khan[2], Regina LaRocque[1], Jennifer Kendall[1], Abu Faruque[2], Cathryn Nagler[1], Edward Ryan[1], Firdausi Qadri[2], Stephen Calderwood[1]
[1]Massachusetts General Hospital, Boston, MA; [2]International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh

Infection with intestinal helminths is common and may contribute to the decreased efficacy of *Vibrio cholerae* vaccines in endemic compared to non-endemic areas. However, the immunomodulatory effects of concomitant intestinal parasitic infection in cholera patients have not been systematically evaluated. We evaluated *V. cholerae* specific immune responses in a cohort of patients with severe cholera. Although there were no significant differences in lipopolysaccharide (LPS)-specific immune responses to *V. cholerae*, helminth infected cholera patients had markedly decreased fecal and serum IgA immune responses to the B subunit of cholera toxin (CTB). These findings remained significant for all classes of helminth infection, even when controlling for potential confounding variables such as age and nutritional status. Although we hypothesized the differential effect on CTB and LPS immune responses was due to T-cell dependent immunomodulatory effects of helminth infection,

we did not find additional evidence to support a classic Th1 or Th2 polarization of the immune response to *V. cholerae* infection related to concomitant helminth infection. Instead, we found that helminth infection had a profound association with decreased mucosal humoral immune response to *V. cholerae*. This has implications for the development of protective immunity in cholera endemic areas.

T.79. Increased Microbial Translocation is Associated with Rapid SIV Disease Progression in Pig Tailed Macaques

Nichole Klatt[1], Levelle Harris[1], Coreen Beaumier[1], David Morcock[2], Brian Tabb[2], Carol Vinton[1], Jeffrey Lifson[2], Bernard Lafont[1], Jacob Estes[2], Jason Brenchley[1]
[1]NIH, Bethesda, MD; [2]NCI, Frederick, MD

Dysfunction of the mucosal immune system is a hallmark of chronic HIV infection. Breakdown of the mucosal barrier is associated with translocation of microbial products across the tight epithelial barrier of the gastrointestinal (GI) tract, which causes systemic immune activation and predicts rates of progression to AIDS. However, the degree to which microbial translocation, as opposed to virus replication, underlies disease progression remains unclear. Here we show that pigtail macaques (PTM), who progress to AIDS during SIV infection much more rapidly than rhesus macaques (RM), have increased microbial translocation prior to SIV infection. In uninfected PTMs, we observe damage to the mucosal integrity, as demonstrated by excessive breaches in the tight epithelial barrier of the GI tract. Furthermore, PTMs have increased levels of plasma LPS compared to either RM ($p=0.005$) or humans ($p=0.008$), and an increased level of CD4+ T cell activation ($p=0.0012$, $p=0.0002$). Following infection with SIV, PTM also have significantly higher microbial translocation-induced immune activation than either RM or humans ($p=0.0012$). These data highlight the importance of microbial translocation in disease progression and suggest that the SIV PTM model is an ideal system to study therapeutic interventions that improve the structural and immunological functions of the GI tract.

T.80. Effect of *Lactobacillus Pentosus* Strain S-PT84 Against Bacterial Infection in Mice

Takayuki Izumo, Masayuki Ida, Toshihiro Maekawa, Yoshinori KItagawa, Yoshinobu Kiso
Suntory Limited, Osaka, Japan

Purpose: *L. pentosus* S-PT84, a plant origin lactic acid bacteria, has been found to enhance immunological functions. In the present study, we elucidated the effects of *L. pentosus* S-PT84 against bacterial infection in mice. Methods: BALB/c mice were administered *L. pentosus* S-PT84 from 7 days before bacterial infection. In three models, 1. Cecal ligation and puncture model, 2. *Salmonella typhimurium* infection model, 3. Cholera toxin revelation model, we examined the effects of *L. pentosus* S-PT84. Results: In *Salmonella typhimurium* infection model, body weight, food and water intake of control group decreased for 7 days after infection. *L. pentosus* S-PT84 could suppressed these phenomena. Salmonella-specific IgA production increased compared to the control group. The numbers of Salmonella in feces, liver, and spleen decreased significantly. Conclusion: *L. pentosus* S-PT84 improved general state and survival rate. The numbers of *Salmonella typhimurium* in the whole organization were also suppressed. These results suggest that *L. pentosus* S-PT84 may be beneficial for prevention against bacterial infection diseases.

T.81. Bacterial Translocation and Inflammatory Response in Gnotobiotic Piglets Infected with *Salmonella Enterica* Serovar Typhimurium or its rfaL- Mutant

Igor Splichal[1], Alla Splichalova[1], Ilja Trebichavsky[1], Daniela Karasova[2], Ivan Rychlik[2]
[1]Institute of Microbiology, ASCR, Novy Hradek, Czech Republic; [2]Veterinary Research Institute, Brno, Czech Republic

Gnotobiotic pigs allow *in vivo* studies of non-pathogenic microbes whose effect would be hidden in complex gut microflora of conventional organisms. One-week-old gnotobiotic piglets were orally infected with 10^8 CFU of virulent wild type LT2 strain of *S. enterica* ser. Typhimurium (wt) or with its rough Ra mutant (*rfaL-*). Bacterial counts in the ileum and bacterial translocation into the organs were compared 24 hours after infection. Plasma concentrations of IL-8 and TNF-alpha were measured by ELISA. Germ-free pigs served as a control group. Ra mutant reached lower numbers of log CFU/mL in the ileum than wt (8 vers. 10) and was absent in the circulation. The log CFU/g of Ra and wt in the mesenteric lymph nodes, lungs, liver and spleen were 5, 3, 1, 1 vers. 6, 4, 4, 6, respectively. Intestinal levels of IL-8 and TNF-alpha in wt-infected pigs were one order higher than in Ra-associated animals, whereas plasma IL-8 and TNF-alpha were found in the former only. Serum sensitive Ra mutant induced local mild cytokine response in the gut only.

T.83. Local and Systemic Inflammatory Response in Gnotobiotic Piglets Associated with Bifidobacteria or *E. coli* Nissle 1917 and Consecutively Infected with Enteropathogenic *E. coli* O55

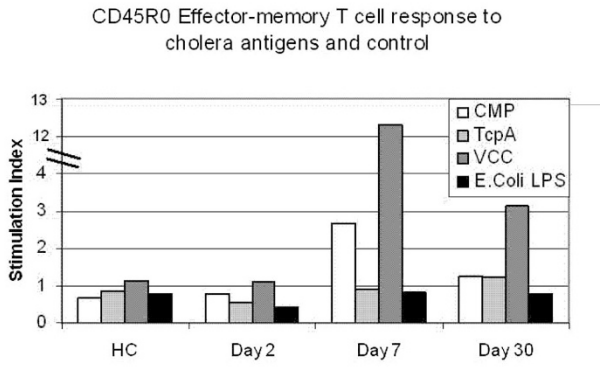
Alla Splichalova[1], Igor Splichal[1], Ilja Trebichavsky[1], Vojtech Rada[2]
[1]Institute of Microbiology, ASCR, Novy Hradek, Czech Republic; [2]Czech University of Life Sciences Prague, Prague, Czech Republic

Germ-free piglets are extremely sensitive to enteropathogenic *E. coli* O55 strain and they succumb to septicaemia in the course of 48 hours after infection. 1-week-old germ-free miniature pigs were orally monoassociated with bifidobacteria for one week (either with a human JKM strain or a pig PR4 strain isolated from faeces) or EcN (*E. coli* Nissle 1917®). Gnotobiotic piglets were subsequently orally infected with 10^8 CFU of O55 for 24 hrs. Control germ-free piglets were infected with O55 only (10^8 CFU for 24 hrs). Cytokine response was measured in

infected piglets. In contrast to very high levels of IL-8, IL-10 and TNF-alpha in small intestine and plasma of O55-monoassociated piglets, all di-associated piglets (with bifidobacteria or EcN for one week and subsequently infected with O55) had low levels of measured cytokines in small intestine, colon and plasma. No significant difference was found between the groups. In spite of this finding, bifidobacteria were not able to protect piglets against O55. On the contrary, EcN-associated piglets survived subsequent infection with O55. Beneficial non-cytokine effect of EcN on the host immune system is discussed.

T.84. Memory T and B Cell Responses to *Vibrio Cholerae* Infection

Ana Weil[1], Mohammad Arifuzzaman[1], Bhuiyan Taufiqur[1], Jason Harris[2], Emily Kendall[1], Azim Hossain[1], Regina LaRocque[2], Aaron Harris[1], Abdullah Tarique[1], Fahima Chowdhury[1], Ashraful Khan[1], Kaylan Banerjee[3], Edward Ryan[2], Firdausi Qadri[1], Stephen Calderwood[2]
[1]*International Centre for Diarrhoeal Disease Research, Dhaka, Bangladesh;* [2]*Massachusetts General Hospital, Boston, MA;* [3]*National Institute of Cholera and Enteric Diseases, Kolkata, India*



Stimulation Index: Lymphoblast count with antigen stimulation divided by blast count without stimulation (SI = 1 indicates stimulation is equal in samples with and without stimulation). *Escherichia coli* LPS was used as a negative control.

Natural *V. cholerae* infection generates a robust memory B cell response that wanes for T cell independent antigens, suggesting that memory B cell responses may be mediated in a T cell dependent manner. We measured the antigen-specific T cell responses of 15 cholera patients and five healthy controls (HC) using the Flow-cytometric Assay of Specific Cell-mediated Immune response in Activated whole blood (FASCIA). On day 7 of infection, the T cell memory-effector response measured by CD4+, CD45R0+ cells stimulated with VCC (*V. cholerae* cytolysin/hemolysin) increased 16-fold versus HC (p=0.04), and also increased for toxin co-regulated pilus subunit A (TcpA, p=0.05) and *V. cholerae* O1 membrane protein preparation (CMP, p=0.01). Significant antigen-specific IgA and IgG serum antibody and antibody-secreting cell responses were observed. TcpA specific IgA memory B cells increased 11-fold between days 2 and 30. Patients with cholera develop a memory-effector T cell response to cholera antigens, and memory B cell activation occurs after T cell population expansion, suggesting that T cells may play an important role in the development and perhaps maintenance of memory B cell responses to T cell dependent antigens. The proliferative response to VCC, whose role in human cholera infection has not been previously studied, was several-fold higher than responses to other cholera antigens.

T.85. Viral Induced T Cell Independent B Cell Activation

Sarah Blutt[1], Margaret Conner[2]
[1]*Baylor College of Medicine, Houston, TX;* [2]*Michael E. Debakey Veterans Affairs Medical Center, Houston, TX*

Many acute viral infections induce early T cell independent polyclonal B cell activation and production of antigen-specific antibody prior to the formation of classical germinal centers. Rotavirus, a well characterized intestinal virus, induces Peyer's patch (PP) T cell independent B cell activation and viral-specific IgA production in mice within 48 hours after viral exposure. We have utilized an *in vitro* culture system to identify important factors required for viral-induced B cell activation. We found that CD11c+ dendritic cells were essential for viral-induced B cell activation and that conditioned medium from virus-treated dendritic cells was sufficient to induce B cell activation indicating that direct contract between the dendritic cell and B cell is not required. PP dendritic cells contained rotavirus antigen and were activated during viral infection *in vivo*, concurrent with B cell activation, suggesting that dendritic cells are important modulators of the B cell response to rotavirus *in vivo* as well as *in vitro*. Our findings suggest that dendritic cells modulate the production of rapid T cell independent pathogen-specific antibody in the PP. Defining how pathogens induce rapid but specific antibody has tremendous potential to define the role of early antibody in limiting viral replication and dissemination and providing protection.