

S5 Towards A Vaccine For Shiga Toxin-Producing *E. coli* (STEC): Protection Against Hemorrhagic Colitis in an Animal Model By Immunization with a Rabbit Enteropathogenic *E. coli* (REPEC) Expressing Truncated Intimin.
T. Agin, L. Johnson, E. Boedecker. Ctr For Vaccine Devel., Univ. of MD, Baltimore

E. coli strain RDEC-H19A is a Shiga toxin (STx)-1-producing derivative of the attaching/effacing (A/E) REPEC strain RDEC-1 which induces hemorrhagic colitis in rabbits resembling human STEC disease. Strategies for STEC vaccination include induction of systemic (or mucosal) anti-toxin immunity or mucosal antibacterial immunity. In previous studies, recovery from intestinal infection with the non-toxin producing RDEC-1 protects against disease induced by toxin-producing RDEC-H19A. To produce an avirulent, but immunogenic vaccine inducing antibacterial immunity, we made an RDEC-1 derivative (M385-RP4) expressing truncated intimin with a stop codon beyond the first of two cysteines at the C terminus of the adhesin. M385-RP4 does not induce A/E lesions *in vitro*, but expresses truncated intimin recognized by anti-intimin antibody in outer membranes. Immunization of rabbits with 2 intragastric doses of M385 (10⁹ CFU) at two week intervals, was well tolerated, and induced measurable systemic antibody responses to intimin as determined by ELISA using intimin-maltose binding protein fusions (4x titer rises in 10/12 animals). After challenge with 10⁹ CFU of RDEC-H19A, immunized animals gained weight (+30 g/day) and had no diarrhea. Non-immunized animals lost weight (-35g/day) and developed watery diarrhea by day five. Fecal shedding of the challenge strain, as shown by cultures from rectal swabs, was significantly reduced in immunized animals by day 2 post-infection. At sacrifice on day seven post-infection, immunized animals were also protected against the development of mucosal edema as demonstrated by analysis of wet/dry weight ratios of intestinal mucosal scrapings. In summary, orogastric immunization of rabbits with a non-adherent, non-toxin producing intimin mutant of RDEC-1 induces systemic anti-intimin antibody and protects animals against weight loss, diarrhea, fecal shedding and mucosal edema following challenge with an STx-1 producing strain.

S7 Topical Application of Antigen to the Skin Surface Rapidly Induces Antigen Specific CD4+ T Cell Responses. T.M. Scharton-Kersten, J. Yu, R. Vassell, C.R. Alving, G.M. Glenn. Department of Membrane Biochemistry, Walter Reed Army Institute of Research, Washington D.C.; IOMAI Corporation, Washington, D.C..

We recently described a non-invasive method of vaccination, transcutaneous immunization (TCI), in which antigen and adjuvant are applied to intact skin for a brief time (≤ two hours). Using bacterial ADP ribosylating exotoxins (i.e. cholera toxin) a potent serum antibody titer (≥1000) develops against both the adjuvant and co-administered antigen. Although antibody induction is an important arm of host immunity, protection against many microbes requires T cell associated defense mechanisms. To evaluate the ability of TCI to elicit T cell responses mice were immunized on the skin at zero, four and eight weeks. Four to twelve weeks after the last immunization, spleen and draining lymph node tissues were removed and the proliferative response to specific and irrelevant antigens assessed. Here we report that TCI with purified experimental and vaccine antigens or pathogen extracts induces CD4+ T cell proliferation. As host immune responses (B and/or T cell) have now been observed with more than thirty antigens, TCI technology appears to be broadly applicable to current vaccine development.

S6 Study of a nasal vaccine against *Neisseria meningitidis* B in a mouse model.

E.N.De Gaspari*, E.F.T.Belo, L.M.C. Coutinho. Seção de Imunologia, Instituto Adolfo Lutz, São Paulo-SP-Brasil

The upper respiratory tract, including nasal mucosa, is the first site of contact with inhaled antigens thus the mucosal barrier provides its first line of defence against microorganisms. Although there is good evidence that locally produced antibodies are more effective at preventing respiratory infections, most vaccines are given parenterally; this type of inoculation stimulates systemic immunity and raises serum antibody levels but induces mucosal immune responses poorly. It is therefore apparent that novel strategies are required to achieve effective local defence. Production of a meningococcal vaccine capable of generating long-lasting immunity in all age groups is still of high priority worldwide. A nasal vaccine using native outer membrane vesicles (NOMV) of the Brazilian *N.meningitidis* epidemic strain B:4:P1.15 was determined in mice. Mice developed serum bactericidal antibodies as well as high levels of specific and cross reactive serum IgA and IgG, as determined by ELISA using whole cells and NOMV of different Brazilian strains. Western blot analysis of mice sera showed IgG and IgA antibody responses to class 1 and 5 protein; additionally, a strong cross reactivity was observed with the peptides of 80kD, 70kD, 50kD and 14kD of Brazil's prevalent strains B:4:P1.15; B:6:P1.6; B:14:NT; B:NT:P1.9; B:17:P1.14; B:19:P1.15 and B:8:P1.6. Monoclonal antibodies from a spleen cell fusion after nasal immunization were obtained: 50% IgG, 20% IgM and 30% IgA. Hybrid clones were identified in a first ELISA screening using whole cells of *N.meningitidis* strains as antigen. The results indicate that meningococcal NOMV contain structures necessary to initiate systemic as well as local mucosal immune responses when presented as a nasal vaccine.
supported by: FAPESP 96/5775-3

S8 Development of an Edible Vaccine to Swine Transmissible Gastroenteritis Virus Using Antigens Expressed in Maize Seed. Joseph M. Jilka^{1*}, Michele Bailey¹, Elizabeth E. Hood¹, D. Bruce Lawhorn, DVM, MS², Ann Kusnadi³, Zivko Nikolov³, and John Howard¹.
¹ProdiGene, College Station, TX, ²College of Veterinary Medicine, Texas A&M University, ³Department of Food Science and Human Nutrition, Iowa State University.

Transmissible gastroenteritis virus (TGEV) of swine is an enteric Coronavirus capable of causing high mortality among neonate swine. Currently we are developing an edible vaccine for swine based on TGEV antigens expressed in maize seed. The coding regions of the spike glycoprotein (S), the matrix protein (M), and the nucleoprotein (N) were optimized for high expression in maize by synthesis of the genes reflecting maize codon usage. These optimized genes were introduced into maize via *Agrobacterium tumefaciens* mediated transformation. We report for the first time high level expression of these TGEV proteins in any recombinant expression system. We will present results from feeding studies using these optimized genes and progress in the development of a TGEV edible vaccine.