

# Fourth Annual Conference

## ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

### P17 Rapid Determination of candidate HLA B\*07- restricted CTL epitopes from the West Nile Virus NY99 genome

Anne S. De Groot<sup>1,2</sup>, Caitlin Saint-Aubin<sup>1</sup>, James Rayner<sup>2</sup>, William Martin<sup>2</sup>

<sup>1</sup>TB/HIV Research Lab, Brown University, Providence RI  
<sup>2</sup>EpiVax, Inc. Providence RI

We applied a bioinformatics (EpiMatrix algorithm) approach to search the West Nile Virus NY99 genome for putative HLA B\*07 restricted cytotoxic T cell (CTL) epitopes. 95 of 3,433 WNV peptides scored above a pre-determined cutoff suggesting that these would be likely to bind to HLA B\*07 and would also be likely candidate CTL epitopes. 16 of the 95 candidate B\*07 ligands and four peptides that were not expected to be ligands based on their EpiMatrixscore were synthesized. Twelve of the sixteen putative HLA B\*07 ligands (75%) were shown stabilize HLA B\*07 molecules on the surface of T2B7 cells. Five of these peptides, IPAGFEPEML (WNV B\*07 0008), RPRWIDARVY (WNV B\*07 0017), RPQRHDEKTL (WNV B\*07 0019), SPHRVPNYNL (WNV B\*07 0020) and RPAADGRVTM (WNV B\*07 0023) bound with much greater affinity to B\*07 *in vitro* than another, previously published, B\*07 epitope GPGHKARVLA (from HIV-1). None of four selected "non-binders" stabilized HLA B\*07 to a significant degree. The binding assays were completed over a four-week period. MHC, ligands identified using this method may be used to screen T cells derived from WNV-exposed individuals for cell-mediated response to WNV, or to develop diagnostic reagents such as tetramers for immunopathogenesis studies and for epidemiological surveillance, and for design of a novel WNV vaccine. This rapid approach to genome analysis illustrates a new paradigm for the application of computational immunology tools in the field of emerging infectious diseases.

### P19 Meningococcal group A Conjugates: Preclinical Studies

A. Bartoloni, F. Norelli, G. Averani, A. Giannozzi, R. Paffetti, S. Berti, R. Rossi, A. Bardotti, S. Ricci, V. Carinci, S. D'Ascenzi, Francesco Di Pisa, Eugenia Gallo, Angela Martino and P. Costantino.  
Chiron Vaccines, Via Fiorentina 1, 53100. Siena, Italy.

**Objective:** The meningococcal polysaccharide vaccines are not effective in infants where the incidence of meningococcal meningitis is higher. In order to improve their immunogenicity, meningococcal polysaccharides or derived oligosaccharides need to be conjugated to protein carriers. The aim of the present pre-clinical study was to evaluate the immunogenicity of two different models of meningococcal group A (MenA) conjugates.

**Methods:** We have prepared two classes of MenA conjugates, one using the native polysaccharide and the other using derived oligosaccharides; in both cases the protein CRM197 was used as carrier. The MenA polysaccharide-CRM197 conjugates were prepared following two procedures: one involving a carbodiimide mediated coupling reaction, the other using reductive amination. The MenA oligosaccharide conjugates were instead prepared according to the following steps: polysaccharide hydrolysis, oligosaccharide sizing by chromatography, oligosaccharide derivatisation to active ester and conjugation to CRM197. The different conjugates were tested for their immunogenicity in mice and guinea pigs. Animal sera were analysed by ELISA.

**Results:** MenA conjugates prepared with the oligosaccharide technology showed a very good immunogenicity. The immunogenicity of the conjugates prepared using the native polysaccharide resulted to be influenced by the conjugation chemistry.

**Conclusions:** The MenA conjugates based on the polysaccharide and oligosaccharide molecular models have been compared in animals. The MenA oligosaccharide-CRM197 conjugate resulted highly immunogenic in mice and guinea pigs. Based on our previous experience with *Haemophilus Influenzae* type b and Meningococcus group C, this model offers also advantages in term of molecular characterisation and manufacturing consistency.

### P18 Characterization of potential vaccine proteins of *Mycobacterium paratuberculosis*.

J. Mullerad<sup>1\*</sup>, Y. Fishman<sup>1</sup>, A. Hovav<sup>1</sup>, I. Michal<sup>1</sup>, R.G. Barletta<sup>2</sup>, H. Bercovier<sup>1</sup>

<sup>1</sup>Department of Clinical Microbiology<sup>1</sup>, Hebrew University, Jerusalem, Israel; Department of Veterinary and Biomedical Sciences<sup>2</sup>, University of Nebraska, Lincoln, NE, USA.

*Mycobacterium paratuberculosis* (MPT) is the etiologic agent of paratuberculosis, a chronic disease of cattle and other ruminants characterized by profuse diarrhea, dehydration and death. The disease is prevalent throughout the world, and exacts a heavy financial toll. At present, the only means of controlling this disease are culling or vaccination. The existing vaccines produce a long lasting local reaction at the point of injection and induce antibodies/DTH reaction that cannot be differentiated from those of naturally infected animals. New acellular vaccines without side effects that allow discrimination between infected and vaccinated animals are necessary to improve the control of this disease. RESEARCH OBJECTIVES: Our goal is to develop an efficient acellular vaccine against paratuberculosis based on protein antigen(s). A prerequisite to achieve this goal is to characterize secreted proteins eliciting cell immunity that can induce a protective immune response. METHODS: isolation, overproduction and purification of MPT recombinant antigens from an expression library in *E. coli*, and characterization of the immune response induced by these antigens in mice (spleen cells proliferation, cytokine production, IgG isotypes). RESULTS AND CONCLUSIONS: Three major secreted antigens were identified: superoxide dismutase (SOD), 85B and 20 kDa protein. The 20 kDa induced a high production of IFN $\gamma$ , IL6 and NO, whereas IL2 and IL10 were barely produced. The proliferation of spleen cells (SI) exposed to this protein was 3 times greater than the control and the IgG1/IgG2a ratio was close to one. 85B induced a similar pattern of lymphokines (even higher IFN $\gamma$  levels) but also induced IL10 and at a lower level IL2. The SI induced by 85B was 5 and the IgG1/IgG2a ratio was 3 or more. The SOD induced a high IL-6 levels and a SI of 3. IgG1/IgG2a ratio was 1.8. The 20 kDa and SOD were the best Th1 inducers and 85B induced both a Th1 and Th2 response with the later slightly less pronounced. Therefore, these proteins are reasonable candidates for the construction of a future acellular vaccine that should induce preferentially a cell mediated immune response.

### P20 DNA-mediated immunization methods with human cytomegalovirus (HCMV) glycoprotein B (gB) for the induction of neutralizing antibodies to HCMV in BALB/c mice

Ye-Jin Kwon, Eun-Suk Park, Sang-Joon Jeon, Chung-Gyu Park, Eung-Soo Hwang\*, and Chang-Yong Cha

Department of Microbiology, Seoul National University College of Medicine, Seoul 150-749, Korea

This study was performed to demonstrate that direct inoculation of DNA vector expressing antigenic determinant to BALB/c mice could elicit the humoral response, especially neutralizing antibody. Immunization was accomplished by inoculating pcGB containing HCMV gB into BALB/c mice intramuscularly, which were divided with 4 groups according to the premedication methods: pure DNA, bupivacaine treatment, cardiotoxin treatment, and gold treatment group. IgG immunofluorescent antibodies appeared at 2 weeks postinoculation, raised peak levels at 7 weeks postinoculation and persisted over 6 months in all group mice. The geometric mean IgG antibody titers at peak level were 1:40.0, 1:165.8, 1:95.6 and 1:65.4 in the premedication group, respectively. Neutralizing antibody was developed, and the percent reduction of plaques in 1:200 diluted sera at 8 weeks postinoculation was 39%, 76%, 66%, and 57%, respectively. This study suggested that DNA vaccine using the gene encoding HCMV gB with bupivacain pretreatment is a candidate method for developing immunity to HCMV.