

**P5** A Nasal Outer Membrane Vesicle (OMV) Vaccine Can Induce Immunological Memory with Strong Booster Antibody Responses

B. Haneberg,<sup>1,2\*</sup> H. Bakke,<sup>1,2</sup> P. Huynh,<sup>1,2</sup> I.L. Haugen,<sup>1</sup> J. Holst,<sup>1</sup> I.S. Aaberge<sup>1</sup> <sup>1</sup>Department of Vaccinology, National Institute of Public Health, Oslo, and <sup>2</sup>Department of Microbiology, Institute of Pharmacy, University of Oslo, Norway.

A vaccine consisting of OMVs from *Neisseria meningitidis* group B was given to mice, either intranasally as four weekly doses without any additional mucosal adjuvant, or once subcutaneously with aluminum hydroxide. Two months later, both groups of animals, as well as unimmunized control mice, received a second course of either intranasal or subcutaneous immunizations. Compared to responses without any pre-immunization, intranasal priming led to significantly higher antibody responses after secondary intranasal as well as subcutaneous immunizations, both regarding IgA antibodies in saliva and extracts of feces, and IgG antibodies in serum. Subcutaneous priming also led to increased antibody responses in secretions and serum after secondary intranasal immunizations. No or only weak secondary antibody responses were observed in secretions with subcutaneous priming and later subcutaneous immunizations. The results indicate that intranasal immunizations can be favourably combined with parenteral vaccinations.

**P6** Identification of a novel outer membrane protein of *Neisseria meningitidis* and its potential as a vaccine candidate.

L.M.C.Coutinho,<sup>1</sup> I.C.A.Scaletsky,<sup>2</sup> E.F.T.Belo,<sup>1</sup> E.N.De Gaspari<sup>1\*</sup> <sup>1</sup>Seção de Imunologia, Instituto Adolfo Lutz; <sup>2</sup>Departamento de Microbiologia Imunologia e Parasitologia, Escola Paulista de Medicina, São Paulo-SP, Brazil.

Adherence is considered to ensure the maintenance of bacteria at the host's mucosal surfaces. We investigated the ability of a cross reactive monoclonal antibody (MAb 8C7Br1) directed against the 50kD peptide of *N.meningitidis* to inhibit bacterial adherence and invasion of HeLa cells. *E.coli* (EPEC and EIEC) strains were grown in 50 ml of TSB for 18 hs at 37°C. Host cells were maintained in Dulbecco's Modified Eagle Medium. The MAb inhibition effect was determined by calculating the percent adherence to or invasion in host cells tested. MAb 8C7Br1 caused 50% and 80% inhibition of adherence and invasion, respectively; the extent of inhibition depended on MAb dilution. With the aid of a fluorescence-activated cell sorter (FACS), we measured antibody bound to surface-exposed epitopes on whole bacteria. FACS analysis using MAb 8C7Br1 and intact *N.meningitidis* or *E.coli* showed that the epitope is expressed on the surface of both bacteria. The NH2-terminal of the 50 kD *N.meningitidis* peptide was sequenced. Immunoblotting of SDS-PAGE resolved proteins using MAb 8C7Br1 showed reactivity with whole cells of all the serogroups, serotypes and subtypes of *N.meningitidis*, *N.lactamica*, *N.gonorrhoeae*, *H.influenzae* type b, *B.pertussis*, *S.typhimurium*, *S.typhi*, *S.flexneri*, *Y.enterocolitica*, and *E.coli* O86, O26, O119, O78, O111, O28, O128, O29 and O157:H7. Future work is being programmed to further determine molecular relationships of antigen bound by MAb 8C7Br1 during inhibition of host cell adhesion and invasion by both *E.coli* strains and the other bacteria studied in the present investigation as well as to directly demonstrate its involvement in pathogenesis. Studies are being conducted in order to express this epitope in an live attenuated bacteria for test as a future oral polyvalent vaccine.

**P7** TRIVALENT SHIGELLAE LIPOPOLYSACCHARIDE VACCINE

Aparin P.G.<sup>1</sup>, Elkina S.I.<sup>2</sup>, Golovina M.E.<sup>1</sup>, Tesheva A.M.<sup>1</sup>, Vaneeva N.P.<sup>2</sup> and L'vov V.L.<sup>1</sup>

<sup>1</sup>National Research Center - Institute of Immunology, <sup>2</sup>I.I.Mechnikov Vaccine and Sera Institute, Moscow, Russia

The trivalent candidate vaccine against shigellosis contains native non-modified S-lipopolysaccharides (LPS's) isolated from three most wide-spread serotypes of *Shigellae*: *Sh.dysenteriae* type1 (*Sh.shigae*), *Sh.sonnei*(phase 1), *Sh.flexneri* type 2a by a combination of microbiological and physico-chemical techniques. Vaccine safety and immunogenicity characteristics have been investigated in preclinical studies on several laboratory animals. *Shigellae* LPS's have shown significantly lower levels of toxicity and pyrogenicity compared with the ordinary Westphal LPS. All three LPS's have proved apyrogenic in standard (Eur.Ph.) rabbit pyrogenicity assay in a dose of 0.025 µg/kg approved by WHO for pyrogenicity control of polysaccharide vaccines. Analysis of LPS's endotoxicity in vivo was carried out under the treatment of randombred white mice by D-galactosamine-hepatotoxic agent, which dramatically increased mice sensitivity to LPS-mediated TNF production. After injection of 10µg LPS *Sh.dysenteriae*, 100 µg LPS *Sh.sonnei*, 1 µg LPS *Sh.flexneri* to D-galactosamine sensitized mice, 100% survival rates have been registered. The dose of safe injection under the same experimental conditions with commercial Westphal-type LPS was only 0.001 µg per mouse, i.e. approximately 1000-times lower. A single immunisation of (CBAXC57B1)F1 mice by *Shigellae* LPS's induced a significant rise of geometric mean serum IgG, IgM antibodies active in the agglutination tests as well. It should be noted that a high O-specific immune response was induced by LPS's doses of 1, 5, 10, 25 µg which were shown to be apyrogenic. Induction of a primary immune response on the background of rational activation of cytokine production by the new LPS's creates the possibility to expect effective immune memory activation and immunogenicity under clinical trials.

**P8** Investigation of Cellular and Humoral Immune Responses to Whole Cell and Acellular Pertussis Vaccines

Catpagavalli Canthaboo, Dorothy K.L. Xing and Michael J. Corbel  
National Institute for Biological Standards and Control, UK

Recent evidence indicates that *B.pertussis* is a facultative intracellular organism. Several studies have shown that *B. pertussis* has the ability to survive intracellularly in mammalian cells *in vitro* and *in vivo*, including macrophages. This implies that cell-mediated immune responses are likely to play a significant role in the elimination of the infection. In the present study, new generation acellular pertussis vaccines compared with old generation whole cell pertussis vaccine for humoral and cellular immune-responses in mice. At the same time, the *in vivo* protective effect of these two types of vaccines was also compared in an improved aerosol challenge model. Macrophages and spleen cells from mice immunised with both types of vaccines induced nitric oxide and the spleen cell cultures induced INF-γ when stimulated with selected antigens *in vitro*. The cultures of macrophages and spleen cells from mice immunised with acellular pertussis vaccines induced less nitric oxide production than cells from mice immunised with whole cell vaccine. However, macrophages and spleen cells from mice immunised with most of the acellular pertussis vaccines tested gave 2-7 fold higher nitric oxide production than the cells from control mice. In general, whole cell vaccine induced lower antibody titres to pertussis toxin and 69 kD antigen than acellular vaccines, but induced a relatively higher antibody response to FHA and Fim 2&3. Comparison in the aerosol challenge model bacterial counts in lungs of mice immunised with one dose of vaccines, whole cell pertussis vaccine showed a better active protection than the acellular pertussis vaccines tested. However, a better protection could be achieved by booster immunisation with acellular vaccine. Our results indicate that acellular pertussis vaccines give relatively stronger humoral immune responses than whole cell vaccine, but the whole cell vaccine induces higher cellular responses than the former. These two types of vaccines may achieve protective immunity through different mechanisms.