

on Vaccine Research

ABSTRACTS OF SUBMITTED POSTER PRESENTATIONS

P45 Polyester microparticles as a vaccine delivery system for brucellosis
C. Gamazo,^{1*} M. Murillo,² M.J. Grilló,³ J. Reñé,² C.M. Marín,³ M.Barberán,³ M.M. Goñi,² J.M. Blasco,³ J.M. Irache.² ¹Dept. Microbiology, ²Dept. Technological Pharmacy, University of Navarra, 31008 Pamplona-Spain. ³Animal Health Unit. SIA-DGA, 50080 Zaragoza-Spain

An antigenic extract of *B. ovis* (HS: lipopolysaccharide, phospholipids and outer membrane proteins) was encapsulated in poly-ε-caprolactone microparticles by the solvent evaporation method, as a vaccine delivery system for ovine brucellosis. These microparticles were injected in mice orally or subcutaneously in order to observe the cytokine pattern, protection against experimental infection with the virulent *B. abortus* 2308 or *B. ovis* PA in Balb/C mice and also the elicited humoral response. The results showed that subcutaneous administration of poly-ε-caprolactone microparticles containing HS extract gave high amounts of IFN-γ and IL-2 but low quantities of IL-4. The vaccine administered either subcutaneously or orally protected mice against *B. ovis* infection. Such protection was similar to that provided by the reference living attenuated *B. melitensis* Rev 1 vaccine that was equally effective by both vaccination routes. By contrast, only the subcutaneous vaccination with microparticles containing HS was as effective as Rev 1 in conferring protection against *B. abortus* infection. The use of free HS or empty microparticles did not produce any protective effect. The antibody isotype immunization suggested the induction of a Th1/Th2 response. Consequently, the data suggests that subcutaneous administration of subcellular vaccine based on poly-ε-caprolactone microparticles are effective and safe against ovine brucellosis

P48 Decreased Non-typeable *Haemophilus influenzae* (NTHi) Infection Following P6 Liposomal Vaccination of Weanling Rats. N. Said¹, W. Ernst², S. Yegiyants¹, L. Song¹, G. Fujii² and J.P. Adler-Moore¹. ¹Cal Poly Pomona, CA. and ²Molecular Express Inc., Los Angeles, CA.

The current *Haemophilus influenzae* vaccine does not protect against NTHi, since NTHi lacks the polysaccharide capsule used in the vaccine. We focused our efforts on the NTHi outer membrane protein P6, coupled to a hydrophobic domain (P6-HD), to promote stable association of the protein with liposomes (LipP6-HD). A rat weanling model was developed for testing the vaccine (n=8-10/group) since it would allow for vaccinations on day 7 and day 21 when the immune system is still developing. The activity of liposomal preparations composed of different amounts of phosphatidylcholine, phosphatidylglycerol, cholesterol, and Lipid A were compared. On day 28, the weanling rats were intraperitoneally challenged with 8.3 Log₁₀ CFU. At peak infection (24h post-challenge), the CSF and blood were collected from the rats, dilutions plated on brain heart infusion agar, supplemented with 20μg/ml of Hemin and 4μg/ml of NAD, incubated 48h at 37°C in 5% CO₂ and then Log₁₀ CFU/ml determined. Serum from 3 rats prior to infection and 5-7 rats, 24h after infection, was collected for detection of agglutination (agg) titers to NTHi. The results showed 80% infection in the non-vaccinated rats with 29%, 57%, 71% and 83% infection for the rats treated with the different liposomal vaccine preparations. The non-vaccinated animals had an average of 7.4 Log₁₀ CFU/ml in the blood and 4.2 Log₁₀ CFU/ml in the CSF. In the LipP6-HD group having the least infection, there were no detectable bacteria in the blood or CSF of 5/7 (71% of the rats). Agg titers were 3-4X higher in the LipP6-HD group having the least infection when compared to rats in the non-vaccinated group. In conclusion, this unique liposomal antigen delivery system markedly decreased infection in neonatal rats, challenged with NTHi, suggesting that this type of vaccine may have potential for controlling bacterial infections.

P46 Induction of CD4 and CD8 T cell responses using antigen encapsulated in gelatin-chondroitin sulfate microspheres.
Ditza Levin*, Ety Ivzada and Rosa Azhari
Dept. Of Biotechnology, Ort Braude College, Karmiel, Israel

New delivery vehicles for vaccination should exhibit adjuvant properties but should also enable triggering of an appropriate protective immunity. In this research we studied the use of microspheres composed of gelatin and chondroitin sulfate as vaccine delivery vehicles. The encapsulation of the antigen and formation of microspheres were achieved using a complex-coacervation process performed under mild, aqueous conditions. The microspheres obtained showed preferential degradation in the vicinity of inflammatory tissue, probably due to enhanced levels of proteases.

Encapsulated antigen (MS-Ag) induced long-term production of both IgG1 (Th2) and IgG2a (Th1) antibodies in mice immunized intraperitoneally (IP). Oral administration of MS-Ag induced a long-term systemic IgG1 response though less intense than that obtained IP or subcutaneously (SC). Ms-Ag sizes 0.25-1 μm were more efficient in producing systemic immunity by oral administration than bigger microspheres. Microspheres sizes 4-10 μm were more effective in IP and SC administration. CD4 T cells derived from mice primed with MS-Ag showed a proliferative recall response to the Ag and secreted a dominant profile of Th1 cytokines.

Using an OVA specific CD4 hybridoma (BO-97-11) and a CD8 one (B3Z) we have shown that macrophages (MQ) and dendritic cells (DC) have the ability to selectively uptake the MS-Ag, process them and present the exogenous antigen on MHC class I and class II molecules. Purified MQ and DC, isolated from mice that were challenged in-vivo with the MS-Ag, also presented the exogenous antigen to CD4 T cells.

Our results indicate that gelatin-chondroitin sulfate microspheres can be used as delivery vehicles for induction of both CD4 and CD8 T cell responses to exogenous antigens. In addition, the composition of the microspheres allows simple binding of antibodies or other targeting moieties to the surface of MS-Ag, thus providing a tool for more specific antigen delivery.

P49 Mucosal administration of totally synthetic lipopeptides without adjuvant induce systemic immune responses
Lbachir BenMohamed*, Yasmine Belkaid*, Estelle Loing#, Karima Brahimi*, Helene Gras-Masse# and Pierre Druilhe*From

the *Biomedical Parasitology Unit, Pasteur Institute, Paris, France. #Chimie des Biomolécules, UMR 8525, Institut de Biologie and Institut Pasteur de Lille, France. ¹Present Address: Ophthalmology Department, Cedars-Sinai Medical Center, Los Angeles, CA 90048, USA. We recently reported that parenteral injection of malaria palmitoyl-tailed peptides efficiently induce without adjuvant B, T_H and CTL responses. We now show that intranasal (I.N.) or sublingual (S.L.) delivery of such lipopeptides induces strong systemic immune responses, as demonstrated by specific Th-cell responses from distant spleen as well as inguinal lymph nodes and by the production of high levels of serum antibodies. Overall, both types of responses were significantly higher than in parallel experiments in which the same lipopeptide was delivered by subcutaneous (S.C.) route. Moreover, we found that dendritic cells (DCs), the principal antigen presenting cell (APC) to encounter antigens within mucosal membranes and the only professional immune-competent cells that are critical for the initiation of immune responses *in vivo*, uptake lipopeptide Ags more efficiently than macrophages (MΦ). Mucosal immunization by lipidated peptides appears therefore as a novel, noninvasive vaccine approach that does not require the use of extraneous adjuvant and, besides cost-effectiveness, has attractive immunological features.