

S25 Clinical Studies of the Tolerance of Intramuscular QS-21 Formulations. C. Kensil¹, E. Jacobson², D. Waite², R. Kammer¹. Aquila Biopharmaceuticals, Framingham, MA, USA, University of Massachusetts Medical Center, Worcester, MA, USA.

QS-21 is an immunological adjuvant that has been used extensively with a wide variety of antigens in human clinical trials. A side effect reported in a few studies is a transient pain upon injection which is reported as an intense stinging or burning sensation. This is observed more frequently if QS-21 is included in a vaccine administered via intramuscular route. For other intramuscularly administered pharmaceuticals, formulation strategies such as pH changes, excipient addition, and local anesthetics have been employed to moderate this effect. We evaluated these strategies with QS-21 and report here the results of randomized, double-blinded studies in which various formulations of QS-21 (50 µg dose) were administered by intramuscular route to healthy volunteers. Perception of pain response was recorded on a 0-10 scale where 0 is no perceived pain and 10 is maximal perceived pain. Each individual received several QS-21 formulations in a blinded fashion and served as his or her own control. The pH of the formulation or the inclusion of aluminum hydroxide did not affect the perceived injection site pain. However, the excipients hydroxypropyl-β-cyclodextrin, polysorbate 80, and the local anesthetic benzyl alcohol were shown to reduce by 50% or more the mean scores for immediate pain on injection associated with QS-21 administration.

S26 Does influenza vaccination protect children against exacerbations of asthma? Piotr Kramarz, F. DeStefano, P. Gargiullo, R.T. Chen and the Vaccine Safety Datalink Team. National Immunization Program, Centers for Disease Control and Prevention, Atlanta, GA, USA.

Objective: Asthma is the most common cause of hospitalization in children. Up to 85% of all asthma exacerbations might be caused by viral infections of upper respiratory tract, including influenza. Little is known about the effectiveness of influenza vaccination in protection against asthma exacerbation. **Methods:** We conducted a historical cohort study of children 1-7 years of age in the Vaccine Safety Datalink - a computerized database containing records on medical encounters of more than 500,000 children enrolled in four large health maintenance organizations. Asthma cases were identified using International Classification of Diseases codes search and asthma medication files. We analyzed the 1995/1996 influenza season (October - April). In children who were vaccinated and had at least one asthma attack, we compared the incidence rates of hospitalization or emergency department visits for asthma exacerbation before and after influenza vaccination. **Results:** We identified 70,753 asthma cases. Influenza vaccine coverage in study children was nine percent. Incidence rate ratio of asthma exacerbation after influenza vaccination versus before vaccination throughout the entire influenza season was 0.65 (95%CI: 0.52-0.80). **Conclusions:** Influenza vaccine effectiveness against asthma exacerbations was 35%. These results should be interpreted with caution, however. The fact that vaccinations on average occurred earlier during the season than the majority of asthma attacks might have been a source of bias. Unspecific effect of the vaccine on the immune system might be another explanation of the observed phenomenon. To adequately assess the effectiveness of influenza vaccination in asthma exacerbation prophylaxis, a clinical trial may be necessary.

S27 Is It Immunologically Feasible to Delay Adult Diphtheria/Tetanus Booster Immunization to Age 50? David W. Scheifele*, Jan J. Ochnio, Margaret Ho and Giselle Lightie. Vaccine Evaluation Center, University of British Columbia, Vancouver, BC, Canada.

Booster doses of diphtheria (D) and tetanus (T) toxoids are recommended 10 yearly in adults but are seldom obtained. Serosurveys show that many Canadian adults over 50 might be susceptible, raising concerns that D outbreaks could reappear here, as in the former Soviet Union. The purpose of this study was to assess responses to a single, mid-life dose of toxoids as has been proposed in the USA to simplify and improve uptake of adult immunizations. Would responses still be adequate 30 years or more after the school-leaving dose? A standard booster dose of Td toxoids (5LF and 2LF, respectively), adsorbed, was given to 71 healthy adults routinely immunized as children and adolescents. In 24 subjects 24-29 years old the booster interval was 10-15 years and in 47 subjects 45-59 years old it was 30-44 years. Serum antitoxin levels were measured just before and one and four weeks after immunization. D antitoxin was measured by toxin neutralization assay in VERO cells and T antitoxin by ELISA. T antitoxin levels were more often < 0.1 IU/ml initially in older subjects (30% vs 4%) and rose more slowly than in younger subjects, but after 4 weeks exceeded 0.1 IU/ml in all subjects. Levels of D antitoxin ≥ 0.01 IU/ml (minimal protection) were present at baseline in 87.5% of younger and 74.5% of older subjects and at 4 weeks post-immunization in 100% of both groups. Fourth-week levels ≥ 0.1 IU/ml (long term protection) were achieved in 81% of older subjects and 100% of younger subjects. We conclude that D & T antitoxin responses are long-lived after childhood immunization and that a single standard dose of Td toxoids is sufficient to restore protective antitoxin levels even after intervals of 30-44 years, supporting the "boost at 50" policy.

S28 Biosynthetic modification of lipid A-associated toxicity and adjuvant activity in *Neisseria meningitidis*: implications for vaccine development Peter van der Ley*, Hendrik Jan Hamstra, Liana Steeghs and Loek van Alphen Laboratory of Vaccine Research, RIVM, Bilthoven. The Netherlands

With the aim of making outer membrane vaccines containing LPS of reduced toxicity, we have investigated the possibility to modify lipid A biosynthesis in *Neisseria meningitidis*. The LpxA protein catalyses the addition of the O-linked 3-OH fatty acid to UDP-GlcNAc, which is the first step in the lipid A biosynthesis pathway. Surprisingly, we have found that a knockout mutation in this gene results in a viable but completely LPS-deficient mutant. In another approach to lipid A modification, we have identified two different homologues of the *htrB/mshB* genes, which in *E. coli* are required for addition of the acyloxyacyl chains. Knockout mutations for both genes were constructed in *N. meningitidis*, resulting in LPS with reduced amounts of laurate. These mutants differ in both structure and biological activity. The immunogenicity of outer membrane complexes (OMCs) of the LPS-deficient mutant derived from strain H44/76 was studied in mice. As measured in both ELISA and bactericidal assay, the immune response was strongly reduced in the absence of LPS. However, addition of external H44/76 LPS to mutant OMCs could restore the immune response. Therefore, a broad panel of adjuvants was tested for their potential to enhance the immunogenicity of LPS-deficient OMCs. AIPO₄, *R. sphaeroides* LPS, monophosphoryl lipid A (MPL) and alkaline-hydrolysed meningococcal LPS showed significantly lower adjuvant activity than wildtype LPS. Adjuvant activity similar to wildtype LPS was found with *E. coli* LPS, meningococcal *icsB* and *rfaC* LPS containing truncated oligosaccharide chains, QuilA and MF59. Interestingly, good adjuvant activity was also found with meningococcal *htrB* LPS, containing penta-acylated lipid A and showing strongly reduced activity in a TNF-α induction assay. Thus, this study demonstrates that the immunogenicity of meningococcal LPS-deficient OMCs can be restored by using other less toxic adjuvants.