Abstracts of Submitted Poster Presentations

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Stable high level expression of foreign antigens from the chromosome of *Salmonella typhimurium* vaccine strains.

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Attenuated *Salmonella* strains are currently being evaluated as live vectors for the delivery of foreign antigens in human and veterinary vaccine trials. In most *Salmonella* delivery systems the foreign antigen is encoded on a multi-copy plasmid, providing high levels of gene expression. However, plasmids encoding the genes for foreign antigens under the control of constitutive promoters are rapidly lost *in vivo*. Several approaches have been used to retain plasmids encoding foreign antigens in *Salmonella in vivo*, either by using selectively expressing promoters or by using balanced lethal systems. Although these approaches have been successful in experimental systems, there are manufacturing and regulatory concerns regarding the use of plasmids encoding foreign antigens in a vaccine. Foreign antigen expression cassettes have been inserted into the *Salmonella* chromosome, offering greater stability than plasmid expression systems, but much lower levels of expression.

We have identified a powerful promoter, co-dependently regulated by anaerobic conditions and cyclic AMP levels that can be inserted in the *Salmonella* chromosome and used to express vaccine antigens. Under anaerobic conditions *in vitro* levels of chromosomally encoded β-galactosidase expressed using this promoter are of a similar order to those obtained from published multi-copy plasmid-based expression systems. In addition, plasmids expressing antigens under the control of this promoter are stable *in vitro* for at least 5 overnight passages in the absence of selection indicating that these plasmid constructs may be stable *in vivo*.

Immunological studies of *Salmonella* strains expressing test antigens under control of this promoter both as a single copy chromosomal insertion and as a plasmid construct will be presented.

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Evaluation of a *Salmonella* Pathogenicity Island-2 (SPI-2) mutant of *Salmonella typhi* as a vaccine vector in mice.

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Increasing knowledge of *Salmonella* pathogenesis has led to the identification of genes that are essential for the *in vivo* growth and survival of these organisms. Introducing defined non-reverting mutations into these virulence genes can lead to the development of novel well-characterised vaccines. Others and we have identified genes within *Salmonella* Pathogenicity Island 2 (SPI-2) critical for the growth of salmonellae within macrophages. SPI-2 mutants of *S. typhimurium* have been shown to be attenuated in conventional *Salmonella*-susceptible BALB/c mice as well as in severely immuno-compromised mice (IFN-γ k.o. and IL-12-treated). Furthermore, SPI-2 mutants strains of *S. typhimurium* are efficient vaccine vectors, eliciting specific antibody to carried antigens when delivered orally to mice. To develop *Salmonella* strains for use as vaccine vectors in humans, a SPI-2 mutation was introduced into a human isolate of *S. typhi*. The SPI-2 mutant strain was transformed with either a plasmid encoding the gene for *E. coli* heat-labile toxin (LTB) or with a plasmid encoding the gene for hepatitis B virus nucleocapsid (HBcAg). Expressions of both antigens are under the control of the constitutive *tac* promoter. Since *S. typhi* is non-infective and poorly/non-immunogenic by the oral route in mice, we utilised the intranasal route to test the immunogenicity of the transformed strains. Intranasal immunization with $1\text{-}2 \times 10^9$ CFUs of *S. typhi* ΔSPI-2/LTB on days 0, 2 and 4 elicited high titers of LT-specific serum IgG as well as LT-specific IgA at a distant mucosal site in the vagina. Sera from *S. typhi* ΔSPI-2/LTB-immunized mice has been shown through *in vitro* analysis to inhibit the binding of LT to GMI-gangliosides (expressed on the surface of gut epithelial cells) and to neutralise the toxicity of LT on GM1-expressing epithelial (Y-1 adrenal) cells. Intranasal immunization with $1\text{-}2 \times 10^9$ CFUs of *S. typhi* ΔSPI-2/HBcAg on days 0, 2 and 4 also led to the production of IgG to the foreign antigen, with high titres of HBcAg-specific IgG present in the sera of all immunised mice. These studies have demonstrated the ability of the SPI-2 mutant *S. typhi* strain to deliver both a bacterial antigen (with adjuvant activity) as well as a particulate viral antigen in an immunogenic form to the murine immune system, highlighting its promise as a vaccine vector for use in humans. We are currently expressing LTB and HBcAg from the chromosome of the SPI-2 mutant *S. typhi* strain under the control of novel *in vivo*-inducible promoters.

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Characterization of protective immune responses elicited by alphavirus replicons expressing the herpes simplex virus type 2 (HSV-2) glycoprotein H (gH)-glycoprotein L (gL) complex.

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Despite the widespread use of effective antiviral therapies, HSV genital disease remains a significant public health problem throughout the world. Vaccines offer the best hope for controlling the spread of HSV infection and limiting disease symptoms. However, induction of broad and sustained protective immunity by prophylactic or therapeutic vaccination remains the key challenge for vaccine success. Vectors based on alphaviruses, such as Sindbis virus (SV) and Venezuelan equine encephalitis virus (VEEV) recently have shown promise as preventative and therapeutic vaccine delivery vehicles directed to infectious diseases and cancer. Previously, we have shown that guinea pigs immunized with VEEV-based replicon particles (VRPs) expressing glycoprotein D (gD) from HSV-2 were protected from intravaginal virus challenge. acute and recurrent disease symptoms were significantly reduced, compared to animal immunized with a placebo control. Here, we describe our continued studies to assess the effect of additional HSV glycoprotein vaccine candidates on infection and disease in the guinea pig model. A unique vector containing two viral promoters was used to generate packaged VRPs expressing both HSV-2 gH (gH2) and gL(gL2). Preliminary results demonstrated that sera from guinea pigs immunized with gH2/gL2-VRPs contain HSV-2 specific neutralizing antibodies. In addition, the severity of acute disease was reduced in vaccinated animals, compared to placebo control animals immunized with VRPs expressing the green fluorescent protein. These studies are continuing, but our results suggest that gH2 and gL2 may also provide some additional protection and should be considered for inclusion in a HSV vaccine formulation. Currently, we are evaluating the immunogenicity and protective effects of gH2/gL2-VRPs in the mouse vaginal challenge model.

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Effects of Conjugation Chemistry on Immunological Reactivity and Epitopes Expressed in type III group B Streptococcal Polysaccharide.

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Conjugation of polysaccharides (PS) to carrier proteins converts the PS from a T-cell independent to a T-cell dependent immunogen. However, PS epitopes can be altered during the conjugation process. Estimation of type III group B *Streptococcus* (GBSIII) PS-specific antibody concentrations by ELISA in human sera is dependent on the coating antigen used. We have investigated effects of several different chemical conjugation methods on the epitopes and immunological reactivity of GBS III PS using ELISA and inhibition ELISA. ELISA antigens used were native and chemically modified GBSIII PS (periodate or cyanylation), and PS from *S. pneumoneae* type 14 (Pn14), which is structurally the same as the GBSIII PS except without the sialic acid residue. The antibody (Ab) samples used included an immune human serum against native GBS III PS, two human monoclonal Ab (Mab) and one mouse Mab to conjugated Pn14 PS, a mouse Mab to native GBSIII PS, and a mouse Mab to conjugated GBSIII PS. The GBSIII Mabs bound strongly to native and modified GBSIII PS but weakly or not all to Pn14 PS. Such binding was readily inhibited by native and modified GBSIII PS, but not by Pn14 PS. Mabs derived using Pn14 PS all bound well to Pn14 PS; some bound more strongly to periodate-treated GBSIII PS than to cyanylation-treated GBSIII PS, while others didn't bind native or modified GBSIII PS. Binding of the Pn14 Mabs were inhibited by Pn14 PS and periodate-modified GBSIII PS, but not by native and cyanylation-modified GBSIII PS. Results of *in vitro* opsonophagocytosis indicated that Mab derived using native or modified GBSIII PS are not opsonic for the Pn14 strain. Mabs derived from Pn14 PS were not opsonic for the GBSIII strain. Our data suggest that native GBSIII PS possesses few cross-reactive epitopes to Pn14 PS. Chemical modification of GBSIII PS increases such cross-reactive epitopes, and periodate treatment more so than cyanylation.