VACCINES / MUCOSAL INFECTION
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OR.9. The Secretory Component of Human Mucosal Non-specific Immunoglobulin A Mediates Inhibition of *Vibrio Cholerae* Biofilm Formation

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Vibrio cholerae, the causative agent of severe diarrheal disease, colonizes the human small intestine and persists in aquatic environments as biofilms. Antigen-specific secretory IgA (sIgA) has been shown to be protective against infection, whereas the effects of non-specific human sIgA on *V. cholerae* colonization or biofilm formation are not fully understood. Non-specific human sIgA inhibited the *V. cholerae* biofilm formation with no reduction in viability under defined biofilm inducing conditions. The inhibition was sIgA-dose-dependent, and was relieved by endonuclease-H treatment that removes high mannose containing oligosaccharides from N-linked glycoproteins. In contrast, non-specific IgM, IgG, or IgA from human serum that lack the secretory component did not inhibit biofilm formation, suggesting that the inhibition was mediated by carbohydrate on the secretory component. Moreover, the ability of *V. cholerae* to colonize the seven day-old infant mouse small intestine was significantly greater in IgA-deficient than in wild type animals, an effect that was mitigated by suckling the IgA-deficient pups on wildtype mothers. These results suggest that non-specific human sIgA, in a secretory component-dependent manner, may play an important role in inhibiting *V. cholerae* colonization and/or biofilm formation in the small intestine.

OR.10. Evaluation of Mucosal B Cell Responses in Shigella-vaccinated Cynomolgus Macaques After Challenge with Wild-type *S. Dysenteriae 1*

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We compared the mucosal B cell responses to *Shigella* antigens in cynomolgus macaques vaccinated with two *Shigella* vaccine candidates and controls after challenge with wild-type *S. dysenteriae* type 1 (wt-Sd1). Groups of 4 monkeys were intragastrically vaccinated with CVD1255 (strain 1617 Δ*stx*AB, Δ*gua*BA, Δ*sen*), or CVD1256 (strain 1617 Δ*stx*A::mLpp-*stx*B, Δ*gua*BA, Δ*sen*), or PBS on day 0 and 28, and challenged with wt-Sd1 on day 56. Two months after the challenge, the animals were sacrificed and cells were isolated from jejunum, ileum, cecum, proximal and distal colon. The numbers of anti-LPS, -IpaB, -IpaC, -IpaD, -VirG and -MxiH IgA and IgG antibody-secreting cells (ASC) were quantified by ELISPOT. Vaccinated monkeys exhibited higher percentages of anti-LPS and anti-IpaB IgA ASC in the mucosal specimens while the specific IgG responses were very low in both vaccinated groups. Interestingly, in a parallel study in which cynomolgus macaques were challenged 3 times with wt-Sd1, strong anti-LPS, as well as anti-IpaB IgA responses were observed; the specific IgG responses were generally lower than IgA. In sum, immunization with attenuated strains of *S. dysenteriae* 1 in cynomolgous macaques appears to be a reliable model to advance vaccine development by enabling the study of mucosal anti-*Shigella* B cell responses following vaccination and challenge.

OR.11. Interferon-γ-dependent Innate Immunity Against Cryptosporidium Parvum Infection in Mice Operates in the Absence of Natural Killer (NK) Cells

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Cryptosporidium parvum infects enterocytes and is commonly the cause of the diarrhoeal disease cryptosporidiosis. Immunocompromised mice that lack T and B lymphocytes partially control infection in part through interferon (IFN)-γ activity. NK cells are the main source of IFN-γ in innate immunity, but a protective role for these cells against Cryptosporidium has not been established. An investigation was made of the role of NK cells in innate immunity to C. parvum employing Rag2-/- mice that lack T and B cells and Rag2-/-γc-/- mice that lack T, B and NK cells. Adult mice developed chronic infections that increased in intensity at a faster rate in Rag-/-γc-/- than in Rag2-/- mice and the Rag-/-γc-/- mice died after several weeks. Neonatal mice initially developed acute infections over about two weeks that were heavier in Rag-/-γc-/- mice, but both strains survived this initial phase of infection. Surprisingly, significant levels of intestinal IFN-γ mRNA were expressed in neonates of both strains. Furthermore, infections were exacerbated in both strains after anti-IFN-γ-neutralising antibody treatment. These results confirm that innate immunity to C. parvum involves IFN-γ and demonstrates a protective role for NK cells. However, the findings with Rag2-/-γc-/- mice also suggest there is a cell type involved in immunity other than NK cells that is a significant source of IFN-γ.

OR.12. Development of a Chlamydia Vaccine for the Koala (Phascolarctos Cinereus)

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Chlamydia pecorum and C. pneumoniae cause urogenital, ocular and respiratory infections in the koala. Infertility and blindness represent a major threat to the species. We characterised the immune response of koalas following subcutaneous vaccination with a multi-subunit vaccine. Three groups of females (n=6) were immunised 3x with a combination of antigens (major outer membrane protein, NrdB and CT512) combined with one of

3 adjuvants. (ISCOMATRIX [ICM], TiterMax gold, 20% Alhydrogel [Alum]). Blood was collected at 2, 6, 14 and 30 weeks for measurement of T cell proliferation, plasma antibody titres and *in vitro* neutralisation activity. Cloacal swabs were collected for antibody determination. Half (n=3) of the animals immunised with TiterMax gold had severe reactions at the vaccination site following the second immunisation. No side effects were seen in animals immunised with ICM or Alum and antigen-specific PBL proliferative responses were still present in these animals at 30 weeks. All animals produced strong serum IgG responses against all 3 antigens that neutralised in vitro infection using multiple strains of Chlamydia. Cloacal swabs also contained antigen-specific IgG. Our data show that subcutaneous immunisation of koalas with a multi-subunit vaccine elicits neutralising systemic and mucosal antibody responses and a systemic cell-mediated response.