

25 Protective Roles of Antibodies Elicited by Lipooligosaccharide Based-Conjugate Vaccines of Nontypeable *Haemophilus influenzae* in Chinchilla Otitis Media Model

J. Sun, J. Chen, X-X. Gu, * NIDCD/NIH, Rockville, MD

Immunization with lipooligosaccharide (LOS) based-conjugate vaccines from nontypeable *Haemophilus influenzae* (NTHi) reduced the incidence of experimental otitis media in chinchillas (Gu, et al. Infect. Immun. 65:4488). In this study, we further analyzed levels and correlations of anti-LOS antibodies (Abs) between sera and middle ear fluids (MEFs), and investigated several biological functions of the antisera to determine what roles of the anti-LOS Abs might play in the protection against otitis media caused by NTHi. After systemic vaccination and middle ear challenge, all vaccinated animals but not the controls showed high titers of anti-LOS Abs in sera and MEFs. There were significant correlations in the levels of anti-LOS IgG+M, IgG and IgA between sera and MEFs ($p < 0.01$). An inverse correlation was found between the levels of serum IgG+M and the bacterial counts in MEFs, and between the levels of MEFs' Abs and the bacterial counts in MEFs at the early stage after challenge. Among 39 vaccinated animals, 44% showed complete protection, 46% of their antisera showed blockage of NTHi adherence to human epithelial cells, 49% demonstrated complement-mediated bactericidal activity against the homologous strain, and 49% showed opsonophagocytic activity against the homologous strain. In contrast, none of the controls (19) showed protection, none of their sera showed positive in blocking bacterial adherence and bactericidal activities, and only 21% showed opsonophagocytosis ($p < 0.05$). These data indicated that systemic anti-NTHi-LOS Abs can transude into middle ear and play a major role in eliminating bacteria through the mechanisms of direct LOS neutralization, blockage of bacterial adherence, complement mediated-bacteriolysis, and opsonophagocytosis.

26 Identification of a Peptide Mimic of a Protective Epitope of Respiratory Syncytial Virus (RSV)

Daniel Chargelegue, Obeid E. Obeid, Shiou-Chih Hsu, * Michael D. Shaw, Andrew N. Denbury, Geraldine Taylor, Michael W. Steward, Department of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, London WC1E 7HT, UK

Objective: To identify peptide mimics of a protective epitope of RSV, the most important cause of bronchiolitis and pneumonia in infants and young children world-wide infection.

Methods: A combinatorial solid-phase peptide library was screened with a protective monoclonal anti-RSV antibody (Mab-19) to identify peptide mimics (mimotopes) of a conserved and conformationally-determined epitope of RSV fusion (F) protein.

Results & Conclusions: Two sequences identified (S1, S2) reacted specifically with Mab-19 when presented as a solid-phase-peptide. Furthermore, after amino-acid substitution analyses, three derived sequences from S1 (S1S; S1K; and S1P) presented as multiple antigen peptides (MAPs) showed enhanced reactivity with Mab-19. The affinity constants of the binding of Mab-19 were 1.19 and $4.93 \times 10^9 \text{ M}^{-1}$ for S1 and S1S, respectively. Immunisation of mice with these mimotopes, presented as MAPs, resulted in the induction of anti-peptide antibodies that inhibited Mab-19 binding to RSV and neutralised viral infection *in vitro*. Following RSV challenge of S1S mimotope-immunised mice, a 98.7% reduction in the titre of virus in the lungs was observed. Furthermore, there was a greatly reduced cell infiltration in the lungs of immunised mice when compared to controls. These results indicate the potential of peptide mimotopes to protect against RSV infection without exacerbating pulmonary pathology.

27 Antibody-Dependent Cell Cytotoxicity (ADCC) Inhibits HIV Replication

Donald N. Forthal, * Gary Landucci, University of California, Irvine College of Medicine

ADCC may play a protective role against viral diseases, and antigens that elicit ADCC antibody (Ab) may be essential components of vaccines. ADCC is generally measured using ^{51}Cr -release assays. However, in viral infections, the anti-viral effect of ADCC is of greater relevance than its ability to lyse target cells. We developed an assay that directly measures the anti-viral effect of ADCC on laboratory and clinical HIV strains.

Methods: Target cells consisting of PBMCs acutely infected with SF₂ or a clinical isolate (R45) were labeled with 10% serum from HIV-infected or uninfected controls. After washing to remove unbound Ab, effector cells (PBMCs from either the target cell donor or a different donor) were added; p24 antigen was then measured in supernatant at various times.

Results: Effector cells from a healthy donor and pooled serum from 5 HIV-infected patients reduced R45 approximately 10-fold compared with serum from an uninfected control. Individual sera differed in their ability to mediate this anti-viral effect. A 30-fold reduction of SF₂ occurred when effector cells were combined with serum from one donor. When targets were incubated with serum alone (in the absence of effector cells) there was no effect; however, effector cells alone (without serum) consistently increased p24 about 2-fold compared with targets incubated in medium alone.

Conclusions: ADCC has a potent *in vitro* anti-viral effect against both clinical and laboratory HIV strains. Directly measuring the anti-viral effect of ADCC should provide a powerful tool for determining mechanisms by which antibody and cytotoxic effector cells inhibit virus and for determining the role of ADCC in viral diseases.