

**9 Optimizing Immune Responses by Coexpression of Costimulatory Molecules with a Hepatitis B DNA Vaccine**

Sowmya Balasubramanian,<sup>1,2\*</sup> Heather L. Davis,<sup>1,2,3</sup> Loeb Research Institute, Ottawa Civic Hospital, Ottawa, Canada; <sup>2</sup>Department of Cellular & Molecular Medicine; <sup>3</sup>Department of Microbiology & Immunology, University of Ottawa, Canada

The use of plasmid DNA encoding antigens has given rise to a novel class of vaccines which may overcome many of the disadvantages associated with classical vaccines. In the present study we examine methods of optimizing immune responses directed against the hepatitis B surface antigen (HBsAg) encoded by a plasmid expression system in BALB/c mice. Interaction of CD80 and CD86 with their receptor CD28/CTLA4 molecules on T cells is known to facilitate progression of the T cell through the cycle of immune activation. We have determined whether coexpression of costimulatory molecules, CD80 or CD86, along with HBsAg from a DNA vaccine can augment HBsAg-specific humoral and cell-mediated responses. Intramuscular or intradermal administration of CD80 or CD86 (10µg dose) with HBsAg (1µg or 10µg) were assessed for total IgG and IgG isotype titres and cytotoxic T-cell lysis. Results from these studies indicate that the immune response generated using DNA vaccines may be augmented by the addition of CD80 or CD86 encoding plasmids.

**10 Construction of a DNA Vaccine for TB Containing Promiscuous T Cell Epitopes**

I. Brichkov,<sup>1</sup> J. A. George,<sup>1</sup> R. G. Whalen,<sup>2</sup> A. S. De Groot,<sup>1\*</sup> <sup>1</sup>TB/HIV Research Laboratory, Brown University School of Medicine, Providence, RI 02912; <sup>2</sup>French National Center for Scientific Research (CNRS), Paris, France

A DNA plasmid encoding the small form of the hepatitis B envelope protein which carries the major surface antigen HBsAg has been cloned. This polypeptide contains all the information needed for HBsAg particle assembly. Such particles are formed and are secreted when the gene for this protein is expressed in a variety of eukaryotic cell types, as detected by the presence of antibodies to the conformational epitope. HBsAg particles are candidates for carrying heterologous epitopes from other pathogens for the purpose of enhancing the immune response to those epitopes.

We have successfully inserted the sequence of a well defined promiscuous T cell epitope derived from MPT 70 within the coding sequence of the carrier protein. This epitope was recognized by 85% of Mtb infected individuals in Providence. The epitope was correctly inserted within the carrier protein sequence, providing a tool for T cell epitope studies in mammalian cell culture systems.

**11 Risk of Allergic Reactions to Gelatin-Containing Vaccines: A Case-Control Analysis of the Vaccine Safety Datalink**

Vitali Pool,\* John Glasser, Paul Gargiullo, Robert T. Chen, the Vaccine Safety Datalink Team, Centers for Disease Control & Prevention, Atlanta, USA

**Objectives:** Recent case-reports suggest that gelatin, used as stabilizer in some vaccines may cause severe allergic reactions. We assessed this association via a case-control study nested within the Vaccine Safety Datalink's (VSD) cohort.

**Methods:** The VSD prospectively links computerized vaccination and medical visit records on ~500,000 children in four Health Maintenance Organizations (HMO). Using ICD-9 codes, cases of selected allergic reactions were identified and categorized by severity and frequency into: anaphylaxis, rare events (urticarias, erythema multiforme, angioneurotic edema), and common events (unspecified allergy, allergic skin reaction, wheezing). Multiple controls were matched to each case by date of birth, HMO and enrollment period. History of vaccinations on the same day as medical visit for cases was compared to that on controls (at the same day of age as matched case's allergic event) using conditional logistic regression, adjusting for confounding by other simultaneously administered vaccines.

**Results:** No increased risk of anaphylaxis was found with any vaccine. Measles-mumps-rubella (MMR) was the only vaccine associated with an increased risk of rare allergies: adjusted odds ratio (OR)=3.3 (95% Confidence Interval [CI]=1.2-8.9). Patients with common allergies were significantly more likely to have received the following vaccines: Influenza (OR=3.4; CI=1.9-6.0), MMR (OR=2.4; CI=1.4-3.2), Varicella (OR=2.1; CI=1.3-3.2), Hepatitis B (OR=2.0; CI=1.4-2.8) and *Haemophilus influenzae* type B (OR=1.7; CI=1.1-2.6).

**Conclusions:** Our findings are consistent with the hypothesis that gelatin may have an etiologic role in allergic vaccine reactions. A laboratory-based study of such patients for antibodies to gelatin is needed to determine whether a safer alternative to gelatin is warranted.

**12 IgE Sensitization to Gelatin: The Possible Role of Gelatin-Containing Diphtheria-Tetanus-Acellular Pertussis (DTaP) Vaccines**

M. Sakaguchi,<sup>1</sup> T. Nakayama,<sup>2</sup> S. Inouye,<sup>2\*</sup> <sup>1</sup>Department of Immunology; <sup>2</sup>Infectious Disease Surveillance Center, National Institute of Infectious Diseases; <sup>3</sup>Kitasato Institute, Tokyo, Japan

Recently we found that most of anaphylaxis to the live virus vaccines including gelatin as a stabilizer might be caused by the gelatin. However, the mechanism how the children were sensitized to gelatin has been unclear.

In Japan, six manufacturers were producing DTaP vaccines. The vaccines from the four companies contain a small amount of gelatin, and the production dose ratio of DTaP vaccines with gelatin to those without gelatin has been 75% to 25%. Because both alum and pertussis toxoid in the vaccines are known as a potent adjuvant for IgE, we investigated a possibility that the gelatin-containing DTaP vaccines elicit the IgE antibody.

We received serum samples of 87 children who had systemic immediate-type reactions including anaphylaxis to measles, mumps, and rubella vaccines throughout Japan during May 1994 to September 1996. Specific IgE to gelatin in the sera was measured by Pharmacia's CAP method. Of the 87 children, 79 had anti-gelatin IgE. We surveyed the 79 children for DTaP vaccine histories and collected the information (the frequency of injection and manufacturer's name) from 55 children. Of these 55 children, none had received DTaP vaccines free of gelatin, whereas 54 had received gelatin-containing vaccines (one child had not received any DTaP vaccine). Therefore, we conclude that there is a strong relationship between gelatin-containing DTaP vaccine injection and anti-gelatin IgE sensitization.