

**P1 Towards Developing an HIV-1 Vaccine for India: Computer-Driven Prediction and Analysis of CTL Epitopes**

Jean-David Barnea,<sup>1</sup> Robert C. Bollinger,<sup>2</sup> Judith A. George,<sup>1</sup> Bill M. Jesdale,<sup>1</sup> Anne S. De Groot,<sup>1\*</sup> <sup>1</sup>TB/HIV Research Laboratory, International Health Institute, Brown University Medical School, Providence, RI; <sup>2</sup>Division of Infectious Diseases, Johns Hopkins Medical School, Baltimore, MD

The number of individuals infected by HIV in India is expected to exceed 5 million in the next two years. The unusually large geographic diversity of HIV-1 coupled with the allelic variation among the major histocompatibility complex (MHC) class I genes among populations has led to the concept of a region-specific HIV vaccine in which endemic viral strains are used to elicit a protective immune response in immune cells expressing local MHC alleles. The current project involves the identification of HIV-1 sequences isolated in India from the literature, and of the most common MHC types in India. These two sets of information were processed using the EpiMatrix algorithm that predicts peptide binding to MHC, a requisite step of CTL activation. Twenty-eight peptides that were highly conserved (>50% of Indian HIV-1 sequences) and that were highly likely to bind to selected MHC (HLA A\*0201, A\*1101, B7 and B35) were identified. Sixteen of these peptides were synthesized and are being tested *in vitro* using an MHC binding assay. Preliminary assays show that four of seven peptides predicted to bind to HLA-B7 were indeed strong binders. Those shown to strongly bind the MHC class I molecule will be further tested using a CTL assay using lymphocytes from HIV-infected Indians. Ultimately, the region-specific CTL epitopes identified may be incorporated into a regional vaccine strategy designed to address HIV/AIDS in India.

**P2 DNA Vaccination with Co-Administration of Cytokines for Prevention of HIV Infection**

M. Bennett,<sup>1\*</sup> A. Shah,<sup>1</sup> A. Cohen,<sup>1</sup> M. Bagarozzi,<sup>2</sup> J. Boyer,<sup>1</sup> T. Vancott,<sup>3</sup> J. Kim,<sup>1,4</sup> M. Lewis,<sup>5</sup> D. Bix,<sup>5</sup> D. B. Weiner.<sup>1</sup> <sup>1</sup>Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA; <sup>2</sup>Department of Pediatrics, Allegheny University of the Health Sciences, Philadelphia, PA; <sup>3</sup>HIV Laboratory, Henry M. Jackson Foundation, Rockville, MD; <sup>4</sup>Department of Chemical Engineering, University of Pennsylvania, Philadelphia, PA; <sup>5</sup>Department of Retroviral Research, Walter Reed Army Institute of Research, Rockville, MD

DNA inoculation with HIV-1 vaccines has led to specific immune responses in a number of species including macaques and chimpanzees. Furthermore, we have recently observed that such vaccinated animals can be protected from infectious challenge. Initially, macaques were immunized and boosted with DNA and protein with adjuvant or DNA alone. The monkeys were subsequently challenged with a chimeric SIV/HIV virus (SHIV) containing the envelope glycoprotein of HIV-1 and gag/polymerase of SIV-239. We observed the development of cellular as well as humoral responses in these studies. In DNA vaccinated animals, 80% developed HIV envelope-specific CTL responses. In an effort to further optimize the induced immune responses, we have incorporated specific cytokine genes as part of these vaccines. Primates have been immunized with IL-4, and IL-12 and their cellular and humoral responses have been evaluated preliminarily. We will explore whether immunization with HIV-mn envelope and SIV-239 gag/pol DNA alone or with cytokines can generate immune responses to HIV and SIV proteins and provide protection from intravenous infection with the chimeric SIV/HIV virus (SHIV-mn) mentioned above. We have observed that IL-4 can boost humoral responses in a dose-dependent manner. In contrast, IL-12 gene delivery appears to boost cellular rather than humoral responses. These studies can provide valuable information about DNA inoculation and the co-administration of cytokine DNA as a possible route to the development of an effective vaccine for HIV-1.

**P3 DNA Vaccination as Immunotherapy for HIV Infection**

A. Cohen,<sup>1\*</sup> M. Bennett,<sup>1</sup> A. Shah,<sup>1</sup> M. Bagarozzi,<sup>2</sup> J. Boyer,<sup>1</sup> A. Javadian,<sup>3</sup> R. R. MacGregor,<sup>4</sup> R. Ginsberg,<sup>5</sup> R. Ciccarelli,<sup>5</sup> D. Weiner.<sup>1</sup> <sup>1</sup>Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA; <sup>2</sup>Department of Pediatrics, Allegheny University of the Health Sciences, Philadelphia, PA; <sup>3</sup>White Sands Research Center, Coulston Foundation, Alamogordo, NM; <sup>4</sup>Infectious Diseases Division, Department of Medicine, University of Pennsylvania, Philadelphia, PA; <sup>5</sup>Apollon Inc., Malvern, PA

Despite recent advances in the therapy of HIV-infected patients, the need for new strategies for the treatment and especially the prevention of HIV infection remains strong. DNA immunization is one such novel strategy. We have previously reported that immunization with plasmid constructs encoding various HIV-1 proteins induces HIV-specific humoral and cellular immune responses. Immunization with a DNA vaccine encoding the *env* and *rev* proteins of HIV-1<sub>mn</sub> boosted immunity in HIV-infected chimpanzees, and a combination of an *env/rev* and an *gag/pol* DNA vaccine protected HIV-negative chimpanzees from intravenous heterologous HIV-1 challenge [JD Boyer *et al.*, *Nature Med.* (1997), 3:526-32]. We have also reported that our *env/rev* vaccine is safe and immunogenic in humans [RR MacGregor *et al.*, *J Inf. Dis.* (1998), in press]. Based on these initial studies, we have begun to explore ways to enhance HIV-specific immune responses in infected non-human primates and humans, both through the administration of different HIV-1 DNA vaccines, as well as through the coadministration of plasmids encoding cytokines or costimulatory molecules. Results from these studies indicate that our DNA constructs which encode the HIV-1 *gag/pol* and *env/rev* proteins are immunogenic in HIV-infected primates, and that the induced immunity can be further enhanced by the coadministration of cytokine genes. These studies suggest that further evaluation of DNA immunization, as both an immunotherapeutic and preventive approach to HIV infection, is warranted.

**P4 Purified Recombinant Meningococcal NspA Protein Induces the Production of Cross-Reactive Bactericidal and Protective Antibodies in Mice and Monkeys**

B. R. Brodeur,<sup>\*</sup> J. Hamel, C. R. Rioux, D. Martin, *Unite de Recherche en Infectiologie, Ctr. Hosp. Univ. de Quebec, Ste-Foy, Quebec, Canada G1V 4G2*

*Neisseria meningitidis* is a major cause of death and morbidity throughout the world and is responsible for both endemic and epidemic diseases principally meningitis and meningococcemia. The NspA protein was shown to be antigenically highly conserved and present in the outer membrane of meningococcal bacteria where it is accessible to the specific antibodies. In order to investigate the vaccinogenic potential of the NspA protein, we have assessed the bactericidal activity of NspA-specific antibodies and the protection conferred by active and passive immunization in a bacteremia model of infection. NspA-specific monoclonal antibody (MAb) Me-7 was bactericidal against 11 out of the 12 serogroup B and all four serogroup A and C meningococcal strains tested when rabbit and human sera were used as the source of complement. This MAb also reduced the levels of bacteremia in mice challenged with 10 serologically distinct meningococcal strains. As expected, mice challenged with a *nspA* meningococcal mutant strain were not protected by MAb Me-7. Analysis of sera collected from NspA-immunized mice surviving meningococcal challenge revealed the presence of NspA-specific antibodies which were bactericidal against 4 serogroup B strains (subtypes 2a, 4 and 15). Immunization of cynomolgus monkeys with 100 and 200 µg of purified recombinant NspA protein also induced the production of cross-reactive NspA-specific IgG antibodies which were bactericidal against three serogroup B strains (2a, 14 and 15). The IgG fraction purified from these monkey sera also passively protected mice against two serogroup B strains (2a and 15). These results demonstrate that the recombinant NspA protein can induce the production of cross-reactive bactericidal and protective antibodies.