

## PLENARY SESSION 03: B CELL BIOLOGY

## PL03-01

## Development of anti-HIV antibodies in humans with high titers of broadly neutralizing antibodies

*M Nussenzweig*<sup>1</sup><sup>1</sup>Howard Hughes Medical Institute, The Rockefeller University, New York, NY, USA

Antibodies to conserved epitopes on the HIV coat protein can protect against infection in non-human primates, and rare infected individuals show high titers of broadly neutralizing IgG antibodies in their serum, but little is known regarding the specificity and activity of these antibodies. To characterize the memory antibody responses to HIV we cloned 501 antibodies from HIV envelope binding memory B cells from six HIV infected patients with high titers of broadly neutralizing antibodies. The development and selection of these antibodies will be discussed.

## PL03-02

## From antibody to vaccine – a tale of structural biology and epitope scaffolds

*G Ofek*<sup>2</sup>, *J Guenaga*<sup>2</sup>, *K McKee*<sup>2</sup>, *J Mascola*<sup>2</sup>, *D Baker*<sup>1</sup>, *W Schief*<sup>1</sup>, *R Wyatt*<sup>2</sup>, and *P Kwong*<sup>2</sup><sup>1</sup>Department of Biochemistry, University of Washington, Seattle, USA; <sup>2</sup>Vaccine Research Center, NIAID/NIH, Bethesda, MD, USA

The multiple means of immune evasion encoded by the HIV-1 envelope glycoproteins – including immunodominant variable loops, extensive glycosylation and conformational flexibility – have frustrated immunogen design. One potential route around these evasion strategies is (1) to define a site of HIV-1 vulnerability recognized by a broadly neutralizing antibody, (2) to transplant the site/epitope into acceptor scaffolds, which are free of the various forms of immune evasion present on the HIV-1 envelope but manage to replicate the conformation and surface accessibility of the site/epitope, and (3) to use these epitope-scaffold immunogens to elicit the desired antibody. We report our efforts with the broadly neutralizing antibody 2F5.

We use structural and computational biology to transplant the 2F5 epitope into seven acceptor scaffolds. Five of these scaffolds were expressed, folded, found to bind 2F5 with better than 20 nM affinity, and displayed up to 1000-fold antigenic discrimination against serum elicited against the unconstrained epitope. Crystallographic characterization of the epitope scaffold with highest affinity and antigenic discrimination showed good to near perfect structural resemblance when free or 2F5-bound, respectively. Immunizations with the epitope scaffolds, meanwhile, elicited antibodies able to recognize all epitope scaffolds, and the structure of a monoclonal antibody elicited by prime/boost immunization with two epitope scaffolds induced a conformation in the flexible epitope similar to that of the parent 2F5. Neutralization by the elicited antibodies is currently borderline, perhaps a consequence of the lack of membrane attachment required for potent neutralization.

## PL03-03

### Induction and function of the mucosal immune system

*P Brandtzaeg<sup>1</sup>*

<sup>1</sup>University of Oslo, Division of Pathology, Oslo University Hospital, Rikshospitalet, Oslo, Norway

The mucosal immune system provides a first defense line which reduces the need for elimination of penetrating exogenous antigens by proinflammatory systemic immunity. To maintain homeostasis, mucosal immunity employs two layers of adaptive anti-inflammatory mechanisms: (a) immune exclusion provided by secretory antibodies to limit epithelial contact and invasion of microorganisms and other potentially dangerous antigens; and (b) immunosuppression to inhibit overreaction against innocuous luminal antigens. The latter strategy is referred to as 'oral tolerance' when induced via the gut; it depends largely on development of suppressive Treg cells in mesenteric lymph nodes to which mucosal dendritic cells (DCs) carry antigens and become conditioned for tolerance induction. Because Treg cells generally dampen immunopathology, they may also hinder elimination of infectious agents such as HIV. Mucosal immunity is most abundantly expressed in the gut, and the intestinal mucosa contains 80% of the body's activated B cells – terminally differentiated to plasmablasts and plasma cells (PCs). Most mucosal PCs produce dimeric IgA which is exported by secretory epithelia expressing the polymeric Ig receptor (pIgR). Immune exclusion is performed mainly by secretory (S)IgA. Notably, pIgR knockout mice which lack Sigs show increased uptake of food and microbial antigens have a hyper-reactive immune system with disposition for anaphylaxis; but this is counteracted by oral tolerance as a homeostatic back-up mechanism. In the intestine, induction and regulation of mucosal immunity takes place primarily in Peyer's patches together with other parts of gut-associated lymphoid tissue (GALT) and mesenteric lymph nodes. Retinoic acid exerts a positive impact both on differentiation of IgA-producing PCs and their intestinal precursor homing. A complication is the regionalization with regard to migration of mucosal memory/effector B cells to various effector sites. However, after the failure of parenteral AIDS vaccines, there is renewed interest in exploiting mucosal immunity in the prevention of this disease.

## ORAL ABSTRACT SESSION 07: T CELL RESPONSES TO VACCINE

## OA07-01

**HIV-Specific CD8<sup>+</sup> T-cells of vaccinees exhibit proliferative and cytotoxic capacities comparable to those of progressors**

*JE Rood<sup>1</sup>, SA Migueles<sup>1</sup>, AM Berkley<sup>1</sup>, AA Compton<sup>1</sup>, RP Joshi<sup>1</sup>, A Duerr<sup>2</sup>, J McElrath<sup>2</sup>, and M Connors<sup>1</sup>*

<sup>1</sup>NIH, Bethesda, MD, USA; <sup>2</sup>Vaccine and Infectious Diseases Unit, Fred Hutchinson Cancer Research, Seattle, WA, USA

**Background:** HIV-specific CD8<sup>+</sup> T-cells of long-term nonprogressors (LTNP) exhibit extraordinary per-cell cytotoxic capacity. To adapt cytotoxicity assays for vaccine trials, we compared HIV-specific CD8<sup>+</sup> T-cell cytotoxic capacity with proliferation and perforin expression using cells from LTNP, viremic progressors, antiretroviral recipients with <50 HIV RNA copies/ml plasma (R<sub>x</sub><50) and seronegative individuals who had or had not received the Merck Ad5 trivalent vaccine.

**Methods:** HIV-specific CD8<sup>+</sup> T-cell cytotoxic responses to HIV<sub>SF162</sub>-infected CD4<sup>+</sup> T-cell targets were measured at 1 hour by flow cytometric detection of granzyme (Gr) B delivery to live targets or infected CD4 elimination (ICE). Cytotoxicity, IFN- $\gamma$  production, perforin expression, and proliferation of HIV-specific CD8<sup>+</sup> T-cells were examined following a 6-day stimulation with HIV<sub>SF162</sub>-infected CD4<sup>+</sup> T-cell targets.

**Results:** The HIV-specific CD8<sup>+</sup> T-cell cytotoxic responses of vaccinees (medians 16.8% GrB activity, 37.2% ICE) were clearly distinguishable from those of seronegative controls (1.7% GrB activity, p<0.001; 0.3% ICE, p<0.001), but were comparable to those of progressors (16.6% GrB activity, p>0.5; 37.4% ICE, p>0.5). Among vaccinees, those with the protective alleles HLA B\*27, B\*57, or B\*58 tended to have higher responses. Vaccinee responses were significantly less than those of LTNP (50.7% GrB activity, p<0.001; 82.5% ICE, p<0.001). GrB activity and ICE were strongly correlated with HIV-specific CD8<sup>+</sup> T-cell proliferation (R=0.85, p<0.001 and R=0.87, p<0.001, respectively) and perforin expression (R=0.86, p<0.001 and R=0.9, p<0.001, respectively). When measured on a per-cell basis, cytotoxicity of most vaccinees remained at the level of progressors, even with higher effector:target ratios.

**Conclusion:** GrB activity and ICE correlate strongly with CD8<sup>+</sup> T-cell proliferation and perforin expression in expanded cells suggesting these parameters are reasonable surrogate measurements of CD8<sup>+</sup> T-cell-mediated killing requiring fewer cells. GrB activity and ICE of vaccinees were similar to those of progressors, suggesting these low responses might contribute to suboptimal control in cases of HIV infection following vaccination.

## OA07-02

**Adenovirus vectors induce expansion of memory CD4 T cells with a mucosal homing phenotype that are readily susceptible to HIV-1 infection**

*A Benlahrech<sup>1</sup>, J Harris<sup>2</sup>, A Meiser<sup>1</sup>, T Papagatsias<sup>1</sup>, J Hornig<sup>1</sup>, P Hayes<sup>1</sup>, A Lieber<sup>3</sup>, T Athanasopoulos<sup>2</sup>, V Bachy<sup>4</sup>, R Daniels<sup>5</sup>, K Fisher<sup>6</sup>, F Gotch<sup>1</sup>, L Klavinskis<sup>4</sup>, L Seymour<sup>6</sup>, K Logan<sup>1</sup>, R Barbagallo<sup>1</sup>, G Dickson<sup>2</sup>, and S Patterson<sup>1</sup>*

<sup>1</sup>Imperial College, London, United Kingdom (Great Britain);

<sup>2</sup>Royal Holloway University of London, Surrey, United

Kingdom (Great Britain); <sup>3</sup>University of Washington, Seattle, USA; <sup>4</sup>King's College London, London, United Kingdom (Great Britain); <sup>5</sup>National Institute for Medical Research, London, United Kingdom (Great Britain); <sup>6</sup>Hybrid Systems Ltd., Oxford, United Kingdom (Great Britain)

**Background:** In the recently halted human immunodeficiency virus type 1 (HIV-1) vaccine STEP trial individuals that were seropositive for adenovirus serotype 5 (Ad5) showed increased rates of HIV-1 infection on vaccination with an Ad5 vaccine. We undertook a series of ex vivo strategies to address the hypothesis that immunisation of Ad5 seropositive individuals with adenoviral vectors may result in activation, expansion, and trafficking of Ad5-specific memory CD4 T cells to mucosal tissues thereby increasing the number of HIV-1 susceptible targets at the initial sites of infection.

**Methods:** Ad5 and Ad11 antibody titers were measured in 20 healthy volunteers. Dendritic cells (DC) were generated from these individuals, pulsed with replication defective Ad5 or Ad11 and co-cultured with autologous lymphocytes. Cytokine profiles, proliferative capacity and the migration potential of the adenovirus-stimulated memory T cells were measured. The susceptibility of re-stimulated memory Ad-specific T cells to infection with a CCR5-utilising HIV-1 was also assessed by multi-colour flow cytometric analysis and p24 ELISA assays.

**Results:** Stimulation of T cells from Ad5 seropositive but Ad11 seronegative individuals with Ad5, or serologically distinct Ad11 vectors induced expansion of adenovirus memory CD4 T cells expressing alpha 4 beta 7 and CCR9, indicating a mucosal-homing phenotype. CD4 T cell proliferation and IFN-gamma production in response to Ad stimulation correlated with Ad5 antibody titers. In contrast, Ad5 serostatus did not correlate with total cytokine production upon re-challenge with Ad5 or Ad11. Expanded Ad5 and Ad11 memory CD4 T cells showed an increase in CCR5 expression and higher susceptibility to infection by R5 tropic HIV-1.

**Conclusion:** Adenoviral-based vaccination against HIV-1 in individuals with pre-existing immunity against Ad5 may result in preferential expansion of HIV-susceptible activated CD4 T cells that home to mucosal tissues, increase the number of virus targets and lead to a higher susceptibility to HIV infection.

## OA07-03

### Influence of preexisting vaccinia immunity on a DNA/MVA SIV vaccine, decreased cellular immunity but enhanced control of a pathogenic SIV challenge

S Kannanganat<sup>4</sup>, P Nigam<sup>4</sup>, V Velu<sup>4</sup>, P Earl<sup>1</sup>, L Lai<sup>4</sup>, B Lawson<sup>4</sup>, L Chennareddi<sup>4</sup>, R Wilson<sup>2</sup>, P Kozlowski<sup>2</sup>, B Moss<sup>1</sup>, H Robinson<sup>3</sup>, and R. Amara<sup>4</sup>

<sup>1</sup>Laboratory of Viral Diseases, NIH, Bethesda, USA; <sup>2</sup>Louisiana State University Health Sciences Center, New Orleans, USA; <sup>3</sup>Geovax Inc., Atlanta, USA; <sup>4</sup>Yerkes National Primate Center of Emory University, Atlanta, USA

**Background:** The influence of preexisting immunity to viral vectors is a major issue for the development of viral vectored vaccines. Here we report that for a DNA/MVA vaccine, preexisting immunity to vaccinia virus (Dryvax) decreases cellular immune responses but enhances control of an intrarectal SIV challenge.

**Methods:** Three groups of rhesus macaques, eight per group, were studied. The Dryvax-naive and Dryvax-immune groups received the DNA/MVA SIV vaccine (DNA at weeks 0 and 8, and rMVA at weeks 16 and 24). In addition, the Dryvax-immune group received the Dryvax vaccine 1.5 years prior to the DNA prime. The control group did not receive any vaccine. All macaques were challenged intrarectally with SIV251 at 9 months after the final MVA.

**Results:** Following vaccination, the frequency of SIV Gag-specific CD4 and CD8 T cells were 5-10 fold lower in the Dryvax-immune group than the Dryvax-naive group. Despite their low SIV-specific T cell responses, the Dryvax-immune macaques exhibited the best control of SIV challenge with viremia 480-fold lower at peak and 40-fold lower at set point than in the unvaccinated control animals ( $p=0.01$ ). The enhanced control in the Dryvax-immune animals was not restricted to Mamu A\*01+ animals and was strongly associated with reduced colorectal virus at 2 weeks post challenge. Factors that correlated with early colorectal viral control included the magnitude of vaccine-elicited CD4 T cells displaying the CCR5 viral co-receptor, which was dampened in the Dryvax-immune animals; the presence of anti-viral mucosal IgA, which was more frequent in the Dryvax-immune animals, and the avidity of the anti-Env Ab response. The frequency of anti-viral CD8 T cells did not correlate with early colorectal viral control.

**Conclusion:** These results highlight important roles for vaccine-elicited CCR5+ CD4 T cells in augmenting, and mucosal IgA and high avidity anti-Env IgG in restricting the early replication of a colorectal immunodeficiency virus challenge.

## OA07-04 LB

### Immunogenicity of ALVAC-HIV® (vCP1521) and AIDSVAX® B/E prime boost vaccination in RV144, the Thai phase III HIV vaccine trial

MS de Souza<sup>1</sup>, R Trichavaroj<sup>1</sup>, A Schuetz<sup>1</sup>, W Chuenarom<sup>1</sup>, Y Phuang-ngern<sup>1</sup>, S Jongrakthaitae<sup>1</sup>, S Ratto-Kim<sup>2</sup>, S Nitayaphan<sup>1</sup>, L Dally<sup>3</sup>, S Serks-Ngarm<sup>4</sup>, J Tartaglia<sup>5</sup>, D Francis<sup>6</sup>, NL Michael<sup>6</sup>, RM Paris<sup>1</sup>, JH Kim<sup>2</sup>

<sup>1</sup> U.S. Military HIV Research Program/AFRIMS, Bangkok, Thailand; <sup>2</sup>U. S. Military HIV Research Program, Rockville, Maryland, USA; <sup>3</sup>EMMES Corporation, Rockville, Maryland, USA; <sup>4</sup>Ministry of Public Health, Nonthaburi, Thailand; <sup>5</sup>Sanofi Pasteur, Toronto, Canada; <sup>6</sup>Global Solutions for Infectious Diseases, San Francisco, California, USA

**Background:** The Phase III trial of ALVAC-HIV® and AIDSVAX® B/E in Thailand began in October 2003 and concluded in June 2009. Both vaccine candidates express HIV-1 circulating recombinant form (CRF) 01\_AE and subtype B antigens. This study assessed whether the Phase III vaccine lots show immunogenicity comparable to the previous Phase I/II study of the identical immunization regimen.

**Methods:** A list of blinded samples from persons completing all 4 injections with either placebo or vaccine and remained HIV negative at the end of the trial was provided. Peripheral blood mononuclear cells (PBMC) or plasma were tested to CRF 01\_AE and subtype B vaccine antigens in the following validated assays: (1) Interferon-gamma (IFN- $\gamma$ ) ELISpot; (2) IFN- $\gamma$ /interleukin-2 intracellular cytokine staining (ICS); (3) Binding antibody (BAb). ELISpot and ICS assays measured responses to Env (92TH023) and Gag (LAI) peptide pools prior to and 6 months following the completion of immunization. BAb was measured using reciprocal dilution EIA to A244 and MN gp120 and BH10 p24 prior to and at 2 weeks following the completion of immunization.

**Results:** Data will be un-blinded to treatment assignment by October 2009. Analyses of post-injection responses to Env and Gag by ELISpot revealed an overall frequency of 14%, with Env responses (11%) predominating over Gag (5%). The overall frequency of ICS responses to HIV peptides in samples studied to date was 35% and was greater for CD4 (26%) than CD8 (9%) T cells, with responses to Env again predominating; 26% versus 1% Gag for CD4 and 6% Env versus 2% Gag for CD8 T cells. The frequency of BAb responses to p24 was 37% and was identical for CRF01\_AE and MN gp120 (70%).

**Conclusion:** Cellular and humoral immune responses to the ALVAC-HIV® + AIDSVAX® B/E regimen were predominantly to HIV Env and appear similar to those seen in the earlier Phase I/II study.