

Australasian HIV/AIDS Conference 2009 – Rapporteur report

Theme A: Understanding and identifying HIV: Basic Science, Biology and Pathogenesis.

This Theme explored fundamentals of HIV and laboratory based research and practice. Areas of focus included the translation of basic research to clinical and laboratory based practice.

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We acknowledge that we have selected talks that complement our own interests and apologise to anyone whose work is not presented. The reports are grouped into 4 broad themes.

1. Intracellular regulation of HIV replication.

On Wednesday, Emma Tippett (paper 320) presented her characterisation of molecules called Tetraspanins because they weave through the cell membrane four times. Tetraspanins CD9, CD53, CD63 and CD81 are implicated in HIV transmission to monocytes (not T-cells). Flow cytometric studies showed differential expression on CD14+ and CD16+ monocytes, with expression of CD53 and CD63 (but not CD81) being low in HIV patients. Confocal microscopy established that CD53 clusters with fluorescent-labelled HIV in infected cultures. Removal of tetraspanins with siRNA reduced R5 and X4 HIV infectivity, but a fusion assay established that this was not mediated by inhibition of viral entry. Despite their high degree of homology, the interactions between CD81 and HIV appeared different from interactions with CD53 and CD63.

David Harrich (paper 477) described the consequences of arginine methylation of Tat. Tat is an essential regulator affecting many aspects of HIV replication. It works in the nucleus, cytoplasm and even extracellular environment. Its activity is increased by acetylation, phosphorylation and ubiquitination and decreased by methylation at arginine (R) 52 and 53. Methylation of Tat is irreversible and creates a stable (persistent) protein. The methyltransferase PRMT6 increases the stability of Tat (by inhibiting degradation by proteosomes) via methylation, but this did not depend of the basic sequence around R52 or 53.

A presentation by Kazuo Suzuki (paper 371) demonstrates a prolonged HIV-1 transcriptional gene silencing in a T-cell line (MOLT-4) by using a RNA duplex targeting the NF- κ B binding motif of the HIV-1 promoter (shkB), delivered with a retroviral vector. HIV replication was inhibited for at least a year, unless the cells were activated by TNF (ie: via NF- κ B). shkB inhibited HIV-1 transcription and did not alter expression of proteins that are receptors for HIV (CD4, CCR5, CXCR4) or NF- κ B driven cellular proteins. Specificity was demonstrated via the failure of shkB to suppress HIV-2 infection. The effectiveness and specificity of shkB represents an important step in gene therapy.

2. HIV-associated immune activation: Feeding the virus

Strategies to eradicate latent viral reservoirs in resting CD4 T-cells were described by Sharon Lewin and Fiona Wightman (papers 475 and 585). Despite the success of ART, viral replication is not completely halted in HIV-infected individuals. This is believed to be due to the presence of latent reservoirs of HIV-1 in resting and transitional CD4 T-cells which exist in

anatomical sites such as the central nervous system, gastrointestinal tract and the genital tract. Potential therapies which target these latent reservoirs may be effective in eliminating residual virus in people on ART. Class 1 histone deacetylase inhibitors (HDACi) such as Oxamflatin, metacept (MCT)-1 and MCT-3 can induce the production of HIV-1 in latently infected T cell lines leading to apoptosis. They may be useful in eliminating latent reservoirs in HIV-1 infected individuals although further trials are necessary as these drugs have a high level of toxicity.

John Zaunders (paper 283) described gut biopsies from primary HIV patients and chronic infection collected in the PINT trial. Gut homing integrin $\alpha 4 \beta 7$ memory T-cells were depleted from circulation in primary and even more in chronic infection. These are preferentially infected but make only 3% of all memory T-cells so they cant sustain HIV disease alone. Cells purified from the biopsies had low CD4:CD8 ratios – partly because CD8 T-cell counts increase. However absolute counts were possible in 7 patients and these generally declined. There was a weak positive correlation between cells in biopsies and $\alpha 4 \beta 7$ + cells in blood, so this population may prove useful in monitoring infection and damage in the gut mucosa.

CD4 T-cells in the gut are rapidly depleted during acute HIV and SIV infection. Miles Davenport and colleagues (paper 420) examined CD4⁺ T-cell depletion in the lamina propria of the rectum in SIV infected macaques and sooty mangabeys during the first 3 weeks of infection. Interestingly, they observed that the peak in viral load occurred days after the peak rate of depletion in these samples. This contrasted to the peak rate of depletion in other biopsy samples (lymph node, lavage fluid, peripheral blood) which matched the time point at which viral load peaked. This suggests that although the gut is a major site of CD4 T-cell depletion in acute SIV infection, it is not the major source of plasma virus.

Paul Cameron (paper 515) described dendritic cell and monocyte subsets isolated from blood and skin. He assessed the role of C-type lectin expression in the DC/T cell interactions that may govern HIV infection of DC. Results of microarray analysis and RT-PCR showed distinct clustering in amongst skin DC, myeloid DC, plasmacytoid DC and monocytes (CD14⁺ and CD16⁺). C type lectins known to bind HIV were differentially expressed on DC from different sites but were found at low levels on resting DC. These data suggest that once activated, each DC subpopulation has a different potential for T-cell interaction and infection with HIV.

3. Antibodies to HIV

Damien Purcell (paper 522) described neutralising antibodies reactive with HIV envelope proteins (gp140) cloned from viruses isolated before seroconversion. By definition these will include antibody-sensitive strains selected for high binding to CCR5. He reviewed the stages needed to evaluate their relatedness via sequence and predicted structure, and their glycosylation as this will determine whether a vaccine recombinant antigen can mimic a natural infection. Artificial viruses were created to assess the function of these envelope proteins, with the surprising result that several conferred R5X4 tropism. The artificial viruses could all be neutralised, some after addition of sCD4 to prompt the conformational change to gp120 associated with CD4 binding on target cells. The data are consistent with theory that these early viruses have exposed neutralising epitopes and are good immunogens.

Damien (paper 521) then reviewed his work on bovine colostrum as a microbicide. Colostrum (first milk) is a valuable source of antiviral proteins and maternal IgG, IgM and IgA. Damien assessed the protective properties of antibodies in colostrum after vaccination with HIV-1 Env oligomers (either soluble clade A, B, C or a TriMix). Pregnant cows received 3 vaccinations, and seroconverted within after 9 weeks. Clade A, B and C Env-pseudotyped reporter viruses were produced to assess the neutralising capacity of the colostrum antibodies. Strong viral

neutralization was observed and titres were high. Production of a microbicide in this way is commercially feasible at 0.3 cents per dose, as a cow can make 850g antibodies per year!

A dramatic new approach to the identification of a broadly neutralising antibody emerged in a paper by S Jung (paper 465). HIV patients co-infected with GBV-C survive better, even if they have cleared their GBV-C through development of anti E2 antibody. This antibody had strong and broad neutralising capacity against HIV (R5 and X4 isolates), as did a murine anti-E2 MoAb reactive with conformational epitopes. Dr Jung suggested that GBV-C E2 may be incorporated into HIV virions making a target for antibodies and an interesting new vaccine candidate.

4. Responses to ART

Biological determinants of immune reconstitution following ART were described by R Rajasuriar (Paper 467). Patients recruited started ART with <500 CD4 T-cells, had undetectable viral load within 6 months and were genotyped for interleukin-7 receptor alpha (IL7R α). Plasma lipopolysaccharide (LPS), soluble (s) CD14 and IL-7 levels were measured pre-HAART and after 6 months. HAART decreased levels of LPS and sCD14 but were still above normal healthy control levels, while IL-7 levels normalized on HAART. Good CD4 T-cell recovery was associated with baseline CD4 T-cell count, low pre-ART LPS levels and the IL-7R α haplotype GTA. This haplotype carries an allele associated with low soluble IL-7R α production, suggesting an increase in free unbound IL-7 to bind membrane-associated IL-7R α . It was interesting that the findings did not reflect control of plasma viraemia.

Mark Connors (papers 64 and 591) defined qualitative features of an effective immune response to HIV. 50% of sera from viraemic patients had antibodies could neutralise 11/20 HIV isolates and 20% could neutralise 15/20 isolates. Long-term non-progressors (LTNP) rarely had neutralising antibodies, probably because their T-cell responses keep antigen levels low. HIV carrying mutations that block its presentation to CD8 T-cells by HLA-B57 are found in all HLA-B57⁺ patients (normal progressors and LTNP). Moreover all HLA-B57⁺ patients have similar numbers and activation of CD8 T-cells. However normal progressors have defective CD8 T-cell proliferative responses and granzyme B and perforin degranulation when compared with LTNP. Hence really good perforin/granzyme loading of HIV-specific CD8 T-cells may be the best explanation for non-progression.

A presentation by Lichtfuss *et al* (paper 514) addressed why NK cell function does not recover with CD4 T-cell function on ART. Loss of NK cell function was correlated with decreased NK cell signaling. Low FcR γ -dependent signaling and Syk phosphorylation levels were described. These were measured by CD16 cross-linking and flow cytometry, respectively. The role of immune activation was established by expression of CD38 and HLA-DR in T-cells, NK cells and monocytes. The presentation suggested that immune activation was inversely correlated with NK cell signaling, supporting the hypothesis that persistent immune activation leads to reduced FcR γ expression and subsequent inhibition of FcR γ signal transduction pathways and loss of NK function.

Genetics doesn't have a high profile at ASHM but with a simple candidate gene approach can place key genes and proteins in the pathway to a defined outcome. Jacquita Affandi (poster 71) addressed whether cytokine genotype can inform mechanisms of susceptibility to tuberculosis (TB) immune restoration disease and non-tubercular mycobacterial (NTM) infections. TNFA-1031*C did not mark susceptibility to TB in untreated Cambodian HIV patients, but marked susceptibility to an IRD event. TNF alleles did not affect susceptibility to NTM, but IL10-1082*G was associated with NTM disease. The rarity of this allele in ethnic Cambodians may explain its lack on an effect on HIV/TB. IL18-607*G was also associated with susceptibility to NTM. Constance Chew (poster 72) addressed TNF haplotypes that predict sensory neuropathy risk in d4T-exposed HIV patients. She found that haplotype

frequencies are ethnicity determined. She then defined haplotypes that associate with neuropathy in Malay and Chinese patients in a univariate analysis. A simple algorithm based on a patient's *TNFA* haplotype status, age and height effectively predicted neuropathy.