

## 017 - Basic science advances in HIV and HTLV-1

### 017.1 HISTONE DEACETYLASE INHIBITORS ALTER THE ACCUMULATION OF CELL-ASSOCIATED SPLICED HIV MRNA – IMPLICATIONS FOR REACTIVATING THE RESERVOIR

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**Background** Clinical trials in HIV-infected patients on antiretroviral therapy with histone deacetylase inhibitors (HDACi) have demonstrated an increase in cell-associated unspliced (CA-US) HIV RNA, variable changes in plasma HIV RNA and no change in the number of latently infected cells. We aimed to define the effects of latency reversing agents (LRAs) on HIV mRNA splicing.

**Methods** Resting CD4<sup>+</sup> T cells isolated from the blood of HIV-negative individuals were treated with the chemokine CCL19 and infected with wild type HIV<sup>NL4.3</sup> to establish latency (n = 9). Latently infected CCL19-stimulated cells were then cultured with vorinostat, romidepsin, JQ1, romidepsin+JQ1 or PMA/PHA, all in the presence of an integrase inhibitor (I8). Cells and supernatant were harvested at 6, 24, 48, and 72 h. Reverse transcriptase (RT) was quantified in supernatant and CA-US and multiply spliced (MS) HIV RNA were quantified by real time qPCR.

**Results** In latently infected CCL19-treated CD4<sup>+</sup> T-cells, stimulation with PMA/PHA led to a significant exponential increase in both US-RNA and MS-RNA by 72 h and reached a mean fold increase above baseline of 34-fold for US-RNA and 54-fold for MS-RNA (p = 0.0003, p = 0.0005 respectively, relative to DMSO). In contrast, following stimulation with each LRA, there was only a modest increase in CA-US RNA that was not statistically significantly different from DMSO (p = 0.89). MS-RNA increased transiently (mean 1.65-fold change at 6hr with romidepsin) and then significantly declined over time with a reduction to 0.18-fold by 72 h relative to DMSO (p = 0.008 romidepsin compared to baseline) in the absence of any cellular cytotoxicity.

**Conclusions** In this *in vitro* model of latency, PMA/PHA and the potent HDACi romidepsin had strikingly different effects on the accumulation of US-RNA, MS-RNA and virus production. Changes in HIV RNA splicing may limit the efficacy of HDACi in activating latent HIV.

### 017.2 CHARACTERISING CLADE-SPECIFIC VIRUS-HOST INTERACTIONS IN HIV INFECTED CLINICALLY ASYMPTOMATIC AND AIDS PRESENTING SUBJECTS

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**Introduction** The utilised co-receptor is indicative of the clinical progression in HIV infected subjects. Differences in clades are known to impact the outcome of HIV infection. In this study,

we investigated the utilised co-receptor and N-glycosylation sites in clinically asymptomatic and AIDS presenting subjects.

**Materials and methods** A total of 1,538 nucleotide sequences encompassing the hyper-variable V3 loop of HIV-1, from clinically asymptomatic and AIDS presenting subjects were downloaded from the Los Alamos Database, which belonged to clades A, B, C and D of HIV-1. Co-receptor prediction was performed using web-based tools PSSM and (*ds*) Kernel. Numbers of N-glycosylation sites were also calculated using the 'N-glycosite' tool.

**Results** CCR5 was the utilised co-receptor in 97% (n = 200) of asymptomatic individuals of clade A and 96.5% (n = 199) of AIDS presenting subjects. In B-clade, 98.9% (n = 194) subjects in asymptomatic group were CCR5 utilising, and 83.5% of AIDS presenting subjects were CCR5 utilising (n = 163, CXCR4 were 22.3%, n = 47). In C-clade the CCR5 was utilised in 193 subjects (asymptomatic, n = 200), and 142 (AIDS presenting, n = 148) utilised both co-receptors (dual co-tropic), and in D clade the co-receptor utilised in 55% subjects was CCR5, n = 154 (CXCR4 in 45% subjects, n = 126), and 81% (n = 198) AIDS presenting subjects utilised CCR5, and 19% utilised CXCR4. Percentage of subjects exhibiting N-glycosylation sites also varied among clades with decrease in number of sites in some and increase in others, when compared between the two clinical categories.

**Conclusions** Co-receptor switching and addition of N-glycosylation sites does not seem to occur universally in all clades studied. The number of N-Glycosylation sites is also not increased from clinically asymptomatic to AIDS presenting subjects. In conclusion, co-receptor switching (from CCR5 to CXCR4) and increase in number of N-glycosylation sites, which are predictive of disease progression, does to occur in all clades universally, thus indicating clade specific responses.

### 017.3 IL-4/IL-13 INHIBITOR VACCINES INDUCE PROTECTIVE IMMUNITY BY MODULATING INNATE LYMPHOCYTIC, DENDRITIC AND MACROPHAGE CELL SUBSETS AT THE VACCINATION SITE

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**Introduction** We have created two novel poxviral vector-based HIV vaccines that transiently inhibit IL-4/IL-13 activity at the vaccination site (murine IL-13Rα2 or IL-4R antagonist) that induce high avidity HIV-specific CD8 T cells with better protective efficacy. Compared to the IL-13Rα2 adjuvanted vaccine, the IL-4R antagonist adjuvanted vaccine induced not only high avidity CD8 T cells but also excellent gag-specific IgG1 and IgG2a antibody differentiation similar to what has been observed in HIV elite controllers. In this study, how IL-4/IL-13 differentially regulate T and B cell immunity following intranasal fowl poxvirus vector based vaccination were evaluated.

**Methods** BALB/c mice were immunised intranasally with recombinant fowl poxvirus co-expressing IL-13Ra2 or IL-4R antagonist adjuvanted together with HIV antigens and wt BALB/c and IL-4, IL-13 gene knockout (KO) mice with the unadjuvanted HIV vaccine. 24 h to 7 days post vaccination different innate lymphocytic cell (ILC) and antigen presenting cell subsets recruited to the vaccination site were evaluated using multi-colour flow cytometry.

**Results** BALB/c mice that received the IL-13Ra2 and IL-4R antagonist vaccines showed significantly reduced IL-13 expression by ILC2 at the lung mucosae compared to the BALB/c that received the unadjuvanted vaccine ( $p < 0.001$ ). Interestingly, the IL-13Ra2 adjuvanted vaccinated group showed significantly elevated ILC1-like cells expressing IFN- $\gamma$  compared to the IL-4R antagonist vaccine ( $p < 0.0001$ ) or BALB/c mice given the FPV-HIV unadjuvanted vaccination ( $p < 0.001$ ). Furthermore, IL-4 and IL-13 milieu also influenced the dendritic and macrophage cell subsets (i.e. CD11b $^{+}$  CD103 $^{-}$  DC, plasmacytoid DC, alveolar macrophages) recruited to the lung mucosae 24 h post vaccination.

**Conclusion** Our findings suggest that i) the outcome of a vaccine is determined within the first 24 h of vaccination, ii) ILC1-like cells most likely play a role in B cell immunity and iii) ILC2 are the major source of IL-13 that dampens CD8 T cell avidity by altering DC/macrophage recruitment to the vaccination site.

**Disclosure of interest statement** Authors have no conflicts of interests. Work was supported by NHMRC and ACH2 EOI grants.

#### 017.4 DEVELOPMENT AND VALIDATION OF A HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE-1 PROVIRAL LOAD ASSAY

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**Introduction** Human T-cell lymphotropic virus (HTLV-I) infects approximately 20 million people world-wide. Transmission requires cell to cell contact and infection can be acquired through breast milk, exposure to HTLV-1 contaminated blood products or sexual contact with an infected person. The HTLV-I proviral load (PVL) is a strong predictor of the risk of transmission and may also serve as an indicator of those most at risk of acquiring significant complications following infection.

The Australo-Melanesian variant (HTLV-I subtype c) is endemic amongst indigenous communities in Central Australia and demonstrates a highly divergent sequence from other known HTLV-I subtypes. Currently, there are no commercially available HTLV PVL assays and published methods fail to reliably detect HTLV-1c.

**Methods** We developed and validated a quantitative, real time PCR (qPCR) assay, specific for the current circulating strains of HTLV-I. Primers and probes were designed by sequencing the *gag* gene from HTLV-1c samples. A highly conserved region of the *gag* gene which did not cross-react with HTLV-II was chosen.

A dilution series of SP cells which contain 1 copy of the HTLV-I genome, was used for quantification. The standards and specimens were run in parallel throughout the entire extraction and qPCR process, allowing us to eliminate variations due to extraction efficiency, PCR amplification and detection. The albumin gene, was used to determine the number of cells/sample and the PVL expressed as HTLV-I copies/cell.

**Results** We have now fully validated this assay using both clinical specimens and cultured cell lines. Clinical specimens consisting

of buffy coats, whole blood specimens and dry blood spots have been tested on the HTLV-I PVL assay and demonstrated good concordance with results obtained using gold standard serology assays.

**Conclusion** We have developed and validated an assay that can reliably quantify the HTLV PVL which may serve as a predictor of the risk of transmission and disease progression.

**Disclosure of interest statement** The authors and their affiliated organisations have no conflicts of interests. This work has been funded through the NHMRC.

#### 017.5 INCREASED HERPES SIMPLEX VIRUS-2 SHEDDING IN HIV-1 INFECTED PERSONS IS DUE TO POOR IMMUNOLOGIC CONTROL IN BOTH GANGLIA AND GENITAL MUCOSA

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**Introduction** A signature feature of HIV infection is poor control of herpesvirus infections, which reactivate from latency and lead to opportunistic infections. While the general mechanism underlying this observation is deficient CD4 $^{+}$ T-cell function, it is unknown whether increased severity of herpes virus infections is due primarily to poor immune control in latent or lytic sites of infection, or whether CD4 $^{+}$  immunodeficiency leads to more critical downstream deficits in humoral or cell-mediated immunologic responses.

**Methods** Here we compare genital shedding patterns of herpes simplex virus-2 (HSV-2) in 98 HIV infected and 98 HIV uninfected men matched on length of infection, HSV-1 serostatus and nationality.

**Results** We demonstrate that high copy HSV-2 shedding is more frequent in HIV positive men, particularly in participants with CD4 $^{+}$  T-cell count  $<200/\mu\text{L}$ . Genital shedding is more frequent due to higher rate of shedding episodes, as well as a higher proportion of prolonged shedding episodes. Peak episode viral load was not found to differ between HIV infected and uninfected participants regardless of CD4 $^{+}$  T-cell count. We simulated a mathematical model which recapitulated these findings and identified that rate of HSV-2 release from neural tissue increases, duration of mucosal cytolytic immune protection decreases, and cell-free viral lifespan increases in HIV infected participants.

**Conclusion** These results suggest that increased HSV-2 shedding is due to impaired immune function in both latent and lytic tissue compartments, with deficits in both humoral and cell-mediated HSV-2 clearance.

**Disclosure of interest statement** No commercial contributions were received that are relevant to this work.