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⁺ T cells isolated from the blood of HIV-negative individuals were treated with the chemokine CCL19 and infected with wild type HIV^{NL4.3} 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⁺ 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.