

**Methods.** Plasma viral RNA was sequenced from a convenience sample of 90 SM cohort samples, and then analyzed for polymorphisms associated with HLA class I and KIR genotypes. An ADCC assay was employed to detect responses to Env and Vpu peptides. An ELISA-based approach was optimized to identify potential Vpu epitopes. Finally, responders from the ADCC assay were assessed in an ADCVI assay.

**Results.** In keeping with lack of CTL targeting, no HLA class I associated polymorphisms were identified in Vpu. KIR analysis, however revealed evidence of a strong association between KIR2DS1 and a single amino acid at position 14 of Vpu. 59% of HIV-1 sequences derived from KIR2DS1+ individuals encoded a valine (V) at this position whereas the consensus amino acid alanine (A) was found at this position in the majority (76%) of KIR2DS1-individuals. ADCC responses to Env were found in 37% of the SM cohort, with only five subjects also showing responses to Vpu peptides. Plasma from all five Env/vpu responders showed potent inhibition of virus replication, nearing 95%, in the ADCVI assay.

**Conclusion.** We demonstrate a significant association between an activating KIR, KIR2DS1, and a polymorphism at amino acid position 14 of HIV-1 Vpu, which is consistent with selection by Natural Killer (NK) cells expressing this KIR. We also demonstrate Vpu and Env ADCC responses that are associated with potent virus inhibition *in vitro* in responders. These data help to shed light upon the immune selection pressures exerted on the HIV-1 vpu gene and may provide insights into the role of this protein in immune evasion.

**Disclosures.** All authors: No reported disclosures.

### 634. Transcriptional Stimulation of Antiviral Response Components by the Structural and Accessory Human coronavirus OC43 Proteins

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**Background.** In Kuwait, human coronavirus OC43 (HCoV-OC43) causes 25–30% of common cold, and 8.8% of respiratory infections in hospitalised patients. It is also associated with severe respiratory symptoms in infants, elderly, and immunocompromised patients. Our previous results showed that the expression of antiviral genes in human embryonic kidney (HEK) 293 cells is downregulated in the presence of HCoV-OC43 proteins. To understand the role of HCoV-OC43 proteins in antagonizing antiviral responses of the host, we investigated the effect of HCoV-OC43 structural and accessory proteins on the transcriptional activation of interferon-stimulated response element (ISRE), interferon- $\beta$  (IFN- $\beta$ ) promoter, and nuclear factor kappa B response element (NF-kappaB-RE).

**Methods.** HCoV-OC43 ns2a, ns5a, membrane (M), and nucleocapsid (N) mRNA were amplified and cloned into the pAcGFP1-N expression vector, followed by transfection in HEK-293 cells. Two days post-transfection, the cells were co-transfected with a reporter vector containing firefly luciferase under the control of ISRE, IFN- $\beta$  promoter, or NF-kappaB-RE. *Renilla* luciferase vector was used as an internal control for transfection efficiency. Following 24 hours of incubation, the cells were treated with either IFN or tumour necrosis factor (TNF) for 6 hours. Thereafter, promoter activity was assayed using the dual-luciferase reporter assay system. Influenza NS1 protein was used as positive control for antagonism.

**Results.** The transcriptional activity of ISRE, IFN- $\beta$  promoter, and NF-kappaB-RE was downregulated in the presence of ns2a, ns5a, M, or N protein as there was a sharp fall in firefly luciferase levels. Overall, HCoV-OC43 proteins reduced firefly luciferase levels for ISRE and IFN- $\beta$  promoter by at least ten fold, whereas for NF-kappaB-RE the firefly luciferase levels were reduced by at least fivefold.

**Conclusion.** HCoV-OC43 has the ability to block the activation of different antiviral signaling pathways.

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### 635. In HIV-Infected Patients Killing of Latently HIV-Infected CD4 T Cells by Autologous CD8 T Cells Is Modulated by Nef

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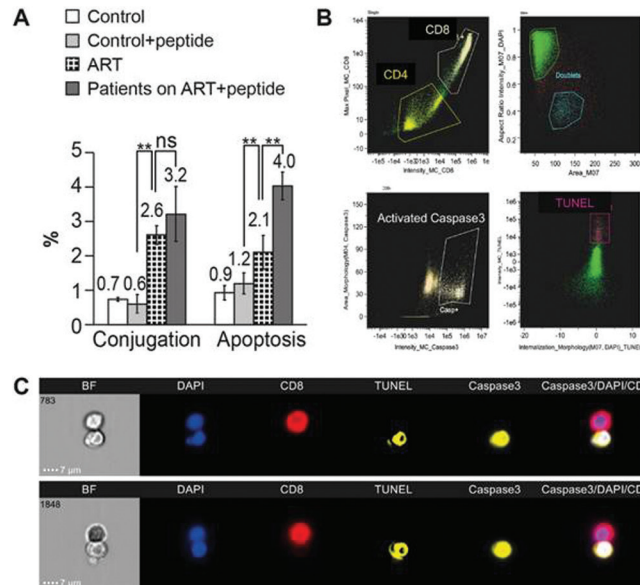
**Background.** The PBMC of HIV-infected patients contain HIV-specific CD8 T cells and their potential targets, CD4 T cells latently infected by HIV. The role of HIV-specific CD8 T cells in the course of HIV infection and the way they affect the virus that resides in the latent reservoir, the resting memory CD4 T cells, is unknown. The association between HIV Nef protein and the cellular ASK1 protein protects the HIV-infected CD4 T cells from killing by CD8 T cells.

**Methods.** CD8 and autologous CD4 T cells procured from PBMC of acute, chronic untreated, treated and AIDS patients were isolated by magnetic beads and co-incubated. Resting memory CD4 T cells (CD25<sup>+</sup>, CD69<sup>+</sup> and HLA-DR<sup>+</sup>) were

isolated from activated CD4 T cells using a two-step bead depletion purification procedure. Formation of CD8-CD4 T-cell conjugates was observed by fluorescence microscopy and *in situ* PCR of HIV LTR DNA. Both conjugation and apoptosis were observed and quantified by imaging flow cytometry (ImageStream) using anti-human activated caspase 3 antibody and TUNEL assay. Formation of immunological synapse was observed by using anti-Perforin, anti  $\gamma$ -tubulin, and anti-LCK antibodies.

**Results.** Following co-incubation we observed that CD8 T cells conjugate with and induce apoptosis of autologous CD4 T cells. In patients with acute infection or AIDS the conjugation activity and apoptosis were much higher compared with chronic HIV-infected patients. In patients on anti-retroviral therapy (ART) low grade conjugation of CD4 T cells was observed by fluorescence microscopy ( $2.3 \pm 0.3\%$ ), by *in situ* PCR of HIV DNA ( $3 \pm 0.6\%$ ) and by ImageStream analysis ( $2.5 \pm 0.5\%$ ). After co-incubation with autologous CD8 T cells  $2.1 \pm 0.4\%$  of the CD4 T cells procured from patients on ART were undergoing apoptosis. Resting memory CD4 T cells were conjugated ( $1.9 \pm 0.3\%$ ) and killed ( $2.2 \pm 0.3\%$ ) by autologous CD8 T cells. Delivering a peptide that interferes with the Nef-ASK1 interaction, into the CD4 T cells, resulted in twofold enhancement of their apoptosis by the autologous CD8 T cells (from  $2.1 \pm 0.5\%$  to  $4.0 \pm 0.4\%$ ), with no effect on conjugation.

**Conclusion.** CD8 T cells conjugate with and kill HIV-infected CD4 T cells throughout the course of HIV infection. We suggest that Nef inhibition may result in the elimination of the latent reservoir CD4 T cells by CD8 T cells.



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### 636. The Hepcidin-25 and Iron Kinetics During the Acute Phase of Systemic Infection

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**Background.** Hepcidin-25, a central regulator of iron metabolism, can decrease serum iron levels by inhibiting the iron transporter ferroportin. Production of hepcidin-25 in hepatocytes is tightly regulated by various stimulations and is promoted by inflammation via the IL-6 pathway. The role of hepcidin-25 in acute infections has not been fully understood; therefore, we investigated the hepcidin and iron kinetics during the acute phase of systemic infection.

**Methods.** We collected clinical samples of bloodstream infections at various stages and measured plasma hepcidin-25 levels using surface enhanced laser desorption/ionization time-of-flight mass spectrometry. In addition, plasma levels of IL-6, C-reactive protein, procalcitonin, presepsin, lipocalin-2 were measured.

**Results.** In this study, 50 patients (median age: 72 years; 52% males) were included. In the acute phase of infection (first 3 days after onset of symptom), plasma hepcidin-25 levels were rapidly elevated, accompanied with a reduction in serum iron concentration. As the inflammation subsequently resolved and the patients' general condition improved ( $\geq 10$  days after symptom onset), serum hepcidin-25 levels were decreased and serum iron levels were restored. Therefore, hepcidin-25 and iron levels dynamically vary during the acute phase of infection, and the enhanced production of hepcidin-25 due to severe inflammation can precipitate a rapid decrease of serum iron levels. This series of reactions may be regarded as a host defense involving the inhibition of the nutrient requirement of bacteria. In this setting, the iron requirement of bacteria is expected to be increased and the iron uptake of bacteria via iron transporter systems may be activated.