

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.

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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- β (IFN- β) 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- β 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- β 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- β 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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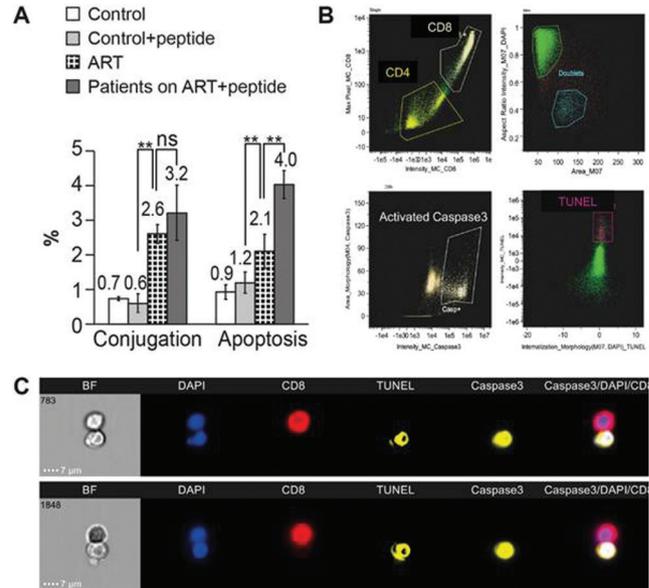
Background. The PBMC of HIV-infected patients contain HIV-specific CD8 T cells and their potential targets, CD4 T cells latently infected by HIV. The role of HIV-specific CD8 T cells in the course of HIV infection and the way they affect the virus that resides in the latent reservoir, the resting memory 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⁻, CD69⁻ and HLA-DR⁻) 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 γ -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 (≥ 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.

Conclusion. During the acute phase of infectious disease with severe inflammation, iron levels were immediately decreased due to enhanced production of hepcidin-25. Understanding of host iron status may be essential for effective use of siderophore cephalosporin, with a unique mechanism of action involving the use of bacterial iron uptake systems.

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637. B-Lactam (BL) Antibiotics Promote an IL-1 β Response in Patients with *Staphylococcus aureus* Bacteremia (SaB)

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Background. BL therapy has been associated with reduced SaB duration compared with non-BL therapy. It has been shown that patients with SaB who fail to generate increased serum IL-1 β are at risk for prolonged SaB (> 4 days duration), a predictor of mortality. This suggests a major role for the IL-1 β host response in prompt clearance of SaB. Furthermore, BL result in reduced peptidoglycan cross-linking, reduced peptidoglycan O-acetylation, and increased alpha-toxin expression, all of which have independently been shown to enhance IL-1 β release. This study aims to show that BL therapy results in a more robust IL-1 β host response compared with non-BL therapy to explain, in part, more rapid SaB clearance.

Methods. Fifty-nine patients (47 MRSA and 12 MSSA) with diverse SaB sources, including endovascular, extravascular (e.g., pneumonia), and catheter-related infections were included. In the first 48 hours, patients were treated with either BL, including oxacillin, cefazolin, or cefazolin ($n = 24$), vs. non-BL vancomycin or daptomycin ($n = 35$). IL-1 β concentrations were determined by ELISA on serum samples obtained on Days 1, 3 and Day 7 after bacteremia onset and compared between groups by Mann-Whitney U test.

Results. Patients in BL and non-BL groups had similar IL-1 β concentrations on Day 1 of bacteremia (median BL 6.1 pg/mL vs. non-BL 2.8 pg/mL, $P = 0.090$). BL-treated patients had significantly higher IL-1 β serum concentrations on Day 3 (median 7.54 mg/mL vs. 1.9 pg/mL; $P = 0.007$) and Day 7 (12.52 pg/mL vs. 1.56 pg/mL, $P = 0.016$) when compared with non-BL-treated patients. BL therapy resulted in 23% and 105% increase in IL-1 β at Days 3 and 7, respectively, while non-BL treatment resulted in 32% and 44% reduction in IL-1 β . The median duration of SaB was similar between BL and non-BL-treated patients (2.5 vs. 2.0 days, respectively, $P = 0.590$).

Conclusion. Given that a lack of inflammasome-mediated IL-1 β production is associated with prolonged SaB, the significant increases in IL-1 β levels in patients treated with BL has important therapeutic implications. Previously observed reduced duration of MRSA bacteremia with the addition of BL to vancomycin may have its basis on enhancing IL-1 β release. A therapeutic regimen of vancomycin or daptomycin in combination with BL to treat MRSA bacteremia and use of BL therapy in MSSA bacteremia is strongly advised to improve outcomes based on these results.

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638. CMV-Specific T-Cell Immune Responses in Older vs. Younger Kidney Transplant Recipients

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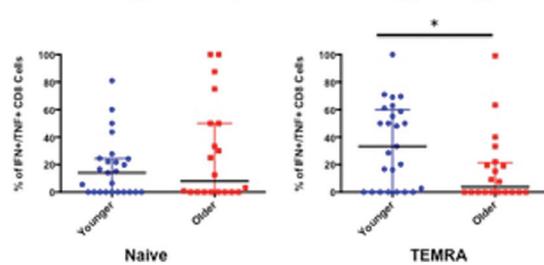
Background. Compared with younger patients on similar immunosuppression regimens, older solid-organ transplant recipients experience increased rates of infection and death, but decreased rates of rejection. The mechanism behind these differences has yet to be defined, but may be related to inflammation driven by CMV infection. The objective of this study was to evaluate older vs. younger solid-organ transplant recipients for CMV-specific T-cell immune responses.

Methods. Peripheral blood mononuclear cells were isolated from 20 older (age 60) and 25 matched younger (ages 30–59) kidney transplant recipients at 3 months after transplantation. Eight recipients were high risk by CMV serology (D+/R–) and 37 were intermediate risk (D–/R+). Overlapping CMV peptide pools were used for stimulation. Intracellular staining to determine cytokine stimulation was performed by multiparameter flow cytometry. Statistical analysis was performed using Jmp Pro 11 software.

Results. There was no association between patient age and CMV risk status ($P = 0.728$). There was no difference between older and younger kidney transplant recipients in release of IFN γ , TNF α , or IL-2 from CD4+ or CD8+ T cells in response to CMV antigen stimulation. However, Older recipients had similar frequencies of CD8+ naive cells but decreased frequency of CD8+ terminally differentiated effector memory CD45RA+ (TEMRA) T cells releasing both IFN γ and TNF α ($P = 0.037$) (figure). Interestingly, development of CMV viremia was associated with a weaker CMV-specific immune response: Patients who had a history of CMV viremia had a decreased frequency of CD8+ TEMRA cells releasing both IFN γ and TNF α ($P = 0.041$).

Conclusion. Older kidney transplant recipients demonstrated a decreased frequency of CMV-specific polyfunctional CD8+ TEMRA T cells. This impaired memory T-cell response to CMV suggests a possible mechanism for the increased vulnerability of older recipients to CMV infection or reactivation, which may in turn worsen age-related immune dysfunction. Furthermore, patients with subsequent CMV viremia had a decreased frequency of CMV-specific polyfunctional CD8+ TEMRA T cells. This finding may explain patient vulnerability to CMV viremia despite modern protocols for antiviral prophylaxis.

Maturation subtype of CMV-specific CD8+ T cells by patient age



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639. Indoleamine 2,3 Dioxygenase, Age, and Chronic Immune Activation in HIV Patients

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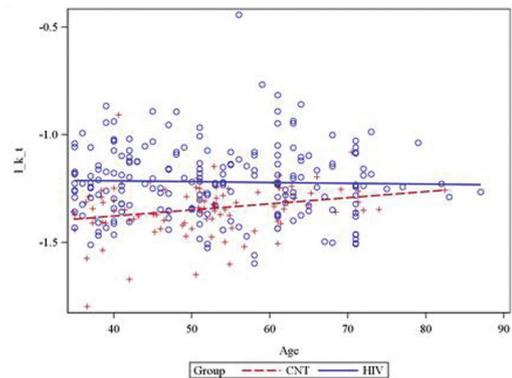
Background. Immune activation complicates HIV despite antiretroviral therapy (ART). Indoleamine 2,3 dioxygenase (IDO) catabolizes tryptophan (T) to kynurenine (K), regulating immune activity. IDO activity increases in HIV patients and non-HIV patients with age. This study examines the relationship of IDO activity, bacterial translocation, and ageing in HIV patients on ART. We hypothesize that increased IDO activity caused by bacterial translocation is a factor in inflammation during aging.

Methods. Samples and data from virologically suppressed HIV patients on ART in specific age strata were obtained from the Centers for AIDS Research Network of Integrated Clinical Systems. Samples and data from age and sex-matched healthy controls were obtained from the Multicenter AIDS Cohort Study and the Women's Interagency HIV Study. The ratio of K to T (K/T) and neopterin were used as indicators of inflammation; 16S ribosomal DNA (16S rDNA) and lipopolysaccharide (LPS) served as markers of bacterial translocation. Log transformation, chi-square tests, t -tests with Satterthwaite adjustment for continuous data, ANOVA, and ANCOVA homogeneity of slopes model were used.

Results. Samples and data from 205 HIV patients and 99 matched controls were analyzed. HIV patients had higher K/T values across all ages. Younger HIV patients had greater K/T values than older healthy controls. Age, sex or race was not associated with differences in K/T. Current CD4 count or CD4 nadir had no association with K/T ratio. For HIV patients, there was an inverse relationship between LPS detection and K/T. For controls, there was no association between LPS and K/T. There was no association between PCR detection of 16S rDNA and K/T ratio in HIV patients or controls. Both groups had positive association between K/T ratio and neopterin.

Conclusion. HIV patients have elevated K/T, even at younger ages, despite virologic control. The main hypothesis that K/T increases with advancing age was not supported in this cohort. Also, unlike other published literature, CD4 nadir, LPS, and 16S rDNA did not correlate with K/T ratio. This study suggests there may be an alternative driver of immune inflammation in well-controlled HIV patients other than bacterial translocation.

Figure 2. Age and K/T ratio.



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