Age Associated Increases in Markers of Microbial Translocation and Inflammation in HIV-1 Infection

Eileen Scully[1,2], Ainsley Lockhart[1], Lisa Huang[3], Yvonne Robles[2], Carlos Becerril[3], Marisol Romero[1], Mary Albrecht[4], Christine Palmer[1], Ronald J Bosch[5], Marcus Altfeld[1,6], Daniel R. Kuritzkes[2], Nina Lin[3,7]

[1]Ragon Institute of MGH, MIT and Harvard, Cambridge, MA, [2]Division of Infectious Diseases, Brigham and Women's Hospital and Harvard Medical School, Boston, MA,[3]Division of Infectious Diseases, Department of Internal Medicine, Massachusetts General Hospital, Boston, MA, [4]Division of Infectious Disease, Beth Israel Deaconess Medical Center, Boston, MA, [5]Harvard School of Public Health, Boston, MA, [6]Heinrich-Pette-Institut, Hamburg, Germany, [7]Division of Infectious Disease, Department of Internal Medicine, Boston University, Boston, MA

Background and Rationale

Antiretroviral therapy (ART) that fully suppresses HIV-1 replication restores immune function and dramatically reduces AIDS-defining complications but does not fully restore health. The current challenge in the care of most HIV-1 infected patients is the management of serious non-AIDS complications that lead to excess morbidity and mortality in this population[1-5]. However, the effects of aging itself on the HIV-1 infected population have complicated the understanding of these epidemiologic observations; distinguishing the influence of HIV-1 from risk factors such as smoking and coinfections is an added challenge[6]. We sought to identify early signs of immune senescence and aging in this population. To address the challenges of confounding comorbid conditions we recruited a well-matched cohort of HIV-1 infected individuals in two age groups and performed detailed phenotypic and functional analyses to identify signatures of aberrant immune activation.

Methods

Patients population
Male HIV+ subjects ≤35 (n=22) or ≥50 years of age (n=23) with at least 6 months of virus suppression on ART were enrolled prospectively at Boston hospitals (MGH, BWH, BIDMC and Fenway Community Health Center). Exclusion criteria: hepatitis B or C, recent active infection, ≥ 5 viral blips, recent immunomodulatory therapy. Uninfected control subjects were recruited from a group of men who have sex with men with self-reported high risk of HIV acquisition as previously described[7].

PBMC studies
Cryopreserved peripheral blood mononuclear cells (PBMCs) were analyzed by flow cytometry for T cell activation, proliferation and exhaustion, monocyte subsets and myeloid and plasmacytoid dendritic cell (mDC and pDC) percentages. PBMCs were cultured with a panel of TLR stimulants LPS, (TLR4); CL097, (TLR7/8); poly I:C (TLR3); ODN 2216, a synthetic CpG (TLR9) (Invivogen) for 20 hours with BFA at time zero, or 5 hours (TLR3, TLR9) and analyzed by intracellular cytokine staining.

Plasma inflammatory profile
Plasma samples were analyzed for: IFNα, IP-10, MCP-1, MIP1β, TNFα, IFNγ, IL-1β, IL-6, and IL-7 with Milliplex assays detected on the Luminex 3D. LPS was measured with the LAL assay and sCD163, sCD14, iFABP and MCP-1 were measured by ELISA.

MCP-1 genotype and mRNA expression
2518 SNP genotype assigned based on genomic restriction digestion as previously described[8]. MCP-1 expression was assessed with qRT-PCR[9] as expression relative to beta actin.

Proviral DNA and cell-associated RNA levels
Total HIV-1 proviral DNA and cell-associated RNA (ca-RNA) was quantified from isolated PBMC using qPCR and qRT-PCR assays[10].

Statistical analysis
Flow cytometry data was analyzed using nonparametric Mann Whitney testing. MCP-1 qRT-PCR data were analyzed as expression relative to beta actin. A 2-$\log_{10}$ statistical outlier was excluded from the final analysis, identified as an outlier by ROUT with a Q value of 0.1% and comparison between groups was determined by Mann-Whitney testing. For plasma levels of sCD14, sCD163 and MCP-1 in the full cohort, quartiles were determined and odds ratios (OR) for the highest quartile were calculated using contingency tables and chi square testing.

Results

Table 1.

Table 1	≤35 (n=22)	≥50 (n=23)
Age [Median (range)]	26 (22-35)	57 (50-73)
Male sex [No (%)]	22 (100)	23 (100)
Ethnicity [No (%)]		
White	10 (45)	15 (65)
Hispanic	4 (18)	4 (17)
Black	3 (14)	4 (17)
Other	5 (23)	0 (0)
$\log_{10}$ plasma HIV-1 viral load, Median (IQR)	5.1 (4.5-5.8)	4.7 (4.4-5.6)
Pretreatment CD4 count, Median (IQR)	320 (250-444)	268 (74-321)
Duration of ART in years, Median (IQR)	2.4 (1.8-3.5)	4.3 (3.1-5.3)
CD4 count at time of sampling, Median (IQR)	659 (558-826)	580 (329-747)
Plasma viral load <50cpm at sampling [No (%)]	22 (100)	23 (100)
CMV seropositivity [No (%)]	12 (92)	17 (94)
Heptitis B and C coinfection	0 (0)	0 (0)
Smoking status [No (%)]		
Current	7 (33)	7 (30)
Former	4 (19)	6 (26)

*For 78% of the cohort, CD4 T cell counts were available from the time of enrollment or within 6 months prior to enrollment date
**For 69% of the cohort, CMV serologic data was available (31 of 45 subjects).
***For 98% of the cohort, smoking data was available

Results

Figure 1:
T cell activation and innate subset distribution

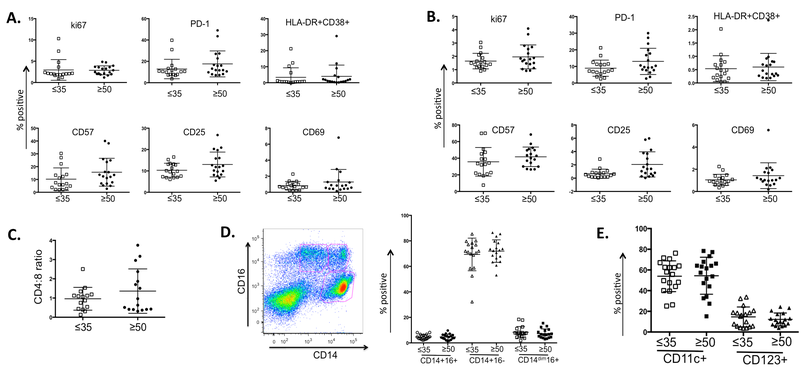


Figure 1. T cell activation and innate subset distribution determined by flow cytometery. $CD4^+$ (**A**) and $CD8^+$ (**B**) T cell proliferation (ki67), exhaustion (PD-1, CD57), and activation (HLA-DR/CD38, CD25, CD69) were comparable between the two groups of HIV-1 infected individuals (*A* and *B*). **C**. The CD4:8 ratio was not significantly different between age groups. HLA-DR positive monocytes were separated into 3 subsets based on expression of CD14 and CD16, and all subsets were represented at similar frequencies (**D**) as were the $CD11c^+$ mDC and $CD123^+$ pDC populations (**E**).

Figure 2:
Intracellular cytokine staining for inflammatory cytokine production after TLR stimulation.

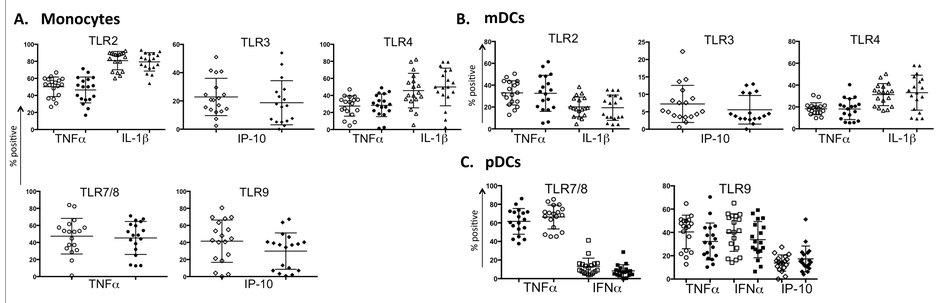


Figure 2. Intracellular cytokine staining for inflammatory cytokine production after TLR stimulation. Cytokine production was measured following a 20 hour stimulation of bulk PBMCs with TLR ligands. No significant differences in cytokine production were seen between ≤35 (open symbols) and ≥50 (filled symbols) in monocytes (**A**), mDCs (**B**), or pDCs (**C**). All data shown represent percent positive after subtraction of background cytokine production in the absence of any added stimulation; values shown only for those cytokines with consistently measurable production

Figure 3:
Analysis of plasma for inflammatory cytokines and markers of microbial translocation.

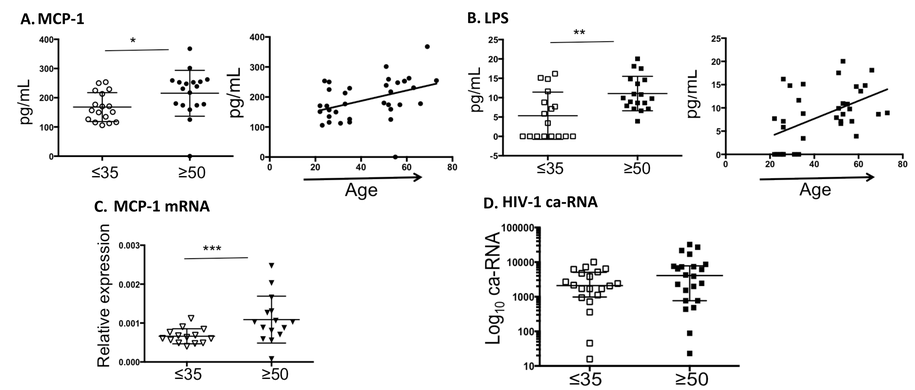


Figure 3. Analysis of plasma for inflammatory cytokines and markers of microbial translocation. Plasma samples from the ≤35 and ≥50 age groups were analyzed for the expression of inflammatory cytokines by Luminex and for levels of LPS, sCD14, iFABP, and sCD163. Significant differences were identified for MCP-1 (**A**), with higher levels of MCP-1 in older individuals by dichotomous comparison (*p= p=0.007, Mann Whitney) and a correlation with age as a continuous variable (Spearman r=0.45, p=0.007). LPS (**B**) also showed a significant relationship to age with a significant difference between groups (**0.005, Mann Whitney) and a correlation with age as a continuous variable (Spearman r=0.53, p=0.001). Bulk RNA from PBMCs matched to the plasma samples was extracted and relative copy number of MCP-1 mRNA was assessed, demonstrating significantly higher expression in the ≥50 age group (**C**, ***p=0.010, Mann Whitney). (**D**) Levels of HIV-1 ca-RNA were not significantly different between the two age strata, although there was a weak association between age and the likelihood of ca-RNA in the upper quartile.

Figure 4:
Relationship of age and HIV-1 infection to plasma markers of inflammation and microbial translocation

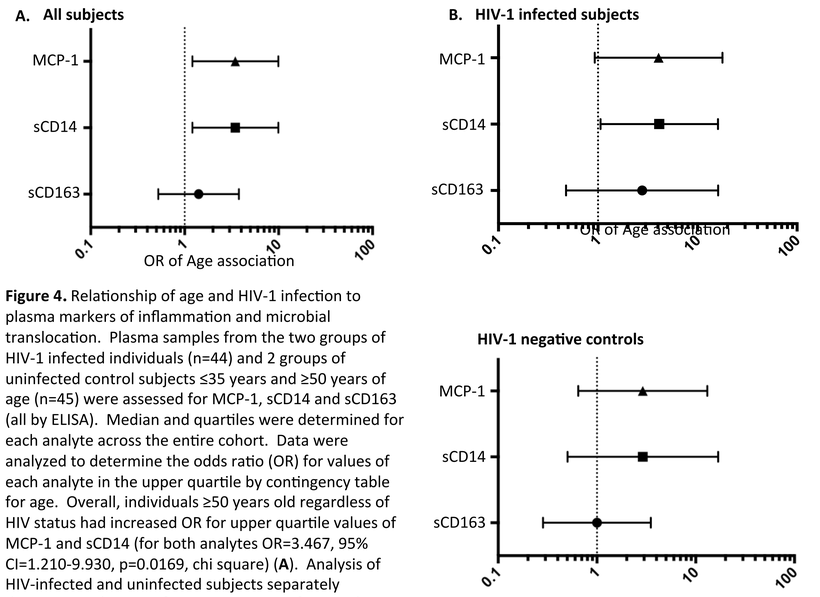


Figure 4. Relationship of age and HIV-1 infection to plasma markers of inflammation and microbial translocation. Plasma samples from the two groups of HIV-1 infected individuals (n=44) and 2 groups of uninfected control subjects ≤35 years and ≥50 years of age (n=45) were assessed for MCP-1, sCD14 and sCD163 (all by ELISA). Median and quartiles were determined for each analyte across the entire cohort. Data were analyzed to determine the odds ratio (OR) for values of each analyte in the upper quartile by contingency table for age. Overall, individuals ≥50 years old regardless of HIV status had increased OR for upper quartile values of MCP-1 and sCD14 (for both analytes OR=3.467, 95% CI=1.210-9.930, p=0.0169, chi square) (**A**). Analysis of HIV-infected and uninfected subjects separately demonstrated no significant association of age with upper quartile values of any of the three analyte (**B**). HIV-1 infected individuals ≥50 years old maintained a higher OR for upper quartile values of sCD14 (OR=4.125, 95% CI 1.061 to 16.04, p=0.0350, chi square)

Conclusions

- This cohort, well matched on virologic characteristics and risk factors including smoking and CMV status, showed few differences in adaptive immune activation.
- Likewise, innate cell distribution and function were comparable between the old and young HIV-1 infected cohorts.
- Despite the comparable measures of cellular immunity, differences in MCP-1 and LPS were identified in plasma with a correlation to MCP-1 mRNA levels. Genotypic analysis of the infected cohort did not show a clear relationship between MCP-1 G allele SNP presence and an increased OR for higher plasma values of MCP-1 (data not shown).
- In a cohort of both infected and uninfected individuals, there was an increased OR for values of MCP-1 and sCD14 to be in the upper quartile for older individuals. Separate analysis of the infected and uninfected groups suggests a stronger relationship for these analytes in the infected cohort.
- Together, the data suggest that exploration of MCP-1 and LPS as a potential early signature of risk for age-related comorbidities is warranted.

Acknowledgements

Work was supported by a CFAR grant to NL and the Ragon Institute Innate Immunity program (MA PI). We are grateful to Ragon Institute clinical and flow cytometry core and to the study subjects.

References

1. Deeks SG, Lewin SR, Havlir DV. The end of AIDS: HIV infection as a chronic disease. Lancet 2013;382:1525-33.
2. Lohse N, Hansen AB, Pedersen G, et al. Survival of persons with and without HIV infection in Denmark, 1995-2005. Annals of internal medicine 2007;146:87-95.
3. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. Lancet 2008;372:293-9.
4. Hasse B, Ledergerber B, Furrer H, et al. Morbidity and aging in HIV-infected persons: the Swiss HIV cohort study. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2011;53:1130-9.
5. Causes of death in HIV-1-infected patients treated with antiretroviral therapy, 1996-2006: collaborative analysis of 13 HIV cohort studies. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2010;50:1387-96.
6. Justice AC. HIV and aging: time for a new paradigm. Current HIV/AIDS reports 2010;7:69-76.
7. Palmer CD, Tomassilli J, Sirignano M, et al. Enhanced immune activation linked to endotoxemia in HIV-1 seronegative MSM. AIDS 2014.
8. Joven J, Coll B, Tous M, et al. The influence of HIV infection on the correlation between plasma concentrations of monocyte chemoattractant protein-1 and carotid atherosclerosis. Clinica chimica acta; international journal of clinical chemistry 2006;368:114-9.
9. Locati M, Deuschle U, Massardi ML, et al. Analysis of the gene expression profile activated by the CC chemokine ligand 5/RANTES and by lipopolysaccharide in human monocytes. J Immunol 2002;168:3557-62.
10. Malnati MS, Scarlatti G, Gatto F, et al. A universal real-time PCR assay for the quantification of group-M HIV-1 proviral load. Nature protocols 2008;3:1240-8.