Sex-Based Differences in Transcriptomic Profiles and HIV Reservoir Correlates

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Background and Rationale

Biological sex impacts multiple aspects of HIV pathogenesis and the host immune response[1]. Women have lower viral loads in the absence of antiretroviral therapy (ART), but progress to AIDS at similar rates[2]. We have previously shown that in the setting of fully suppressive ART, women and men have comparable levels of HIV DNA, but women have lower levels of residual viremia by single copy assay and lower levels of multiply-spliced cell-associated HIV. In the same cohort, we observed lower levels of T cell activation and antigen experience as measured by PD-1 expression in women as compared to men(Scully et al., Abstract 281,CROI 2017, summarized below). Immunologic correlates and gene expression patterns that associate with HIV reservoir size and activity are still under investigation and may highlight potential curative interventions. To identify sex-specific pathways relevant to HIV pathogenesis and cure strategies, we performed transcriptional profiling on this cohort of HIV-positive men and women matched on critical virologic and immunologic factors. Data were analyzed to identify genes and pathways differentially expressed by sex, and gene expression was related to virologic parameters.

Methods

Cohort: Premenopausal women on ART with ≥1 year of viral suppression were prospectively enrolled (n=26) and matched with men (n=26) on age, duration of viral suppression, CD4 count/nadir and unusual clinical phenotypes. All participants were enrolled through the SCOPE cohort at UCSF and provided written informed consent.

Measures of HIV reservoir and immune activation Integrated HIV DNA (iDNA) was measured in resting CD4 T cells. Cell associated (CA) multiply spliced (ms) and unspliced (us) HIV RNA in resting CD4 T cells was measured and normalized to 18S RNA input. T cells were phenotyped by flow cytometry.

Transcriptional profiling and analysis: Peripheral blood was collected in PAXgene™ tubes. RNA was isolated and sequenced with the 3' digital gene expression platform (Broad Institute, Cambridge, MA). Data was preprocessed: trimming (Trimmomatic v 0.33), alignment (STAR v 2.2.2a, sparse indexing, Human genome GRCh38 2015-2016), counting (HTSeq v 0.6.1, output=exon counts, gene counts, transcript counts). Data quality was good, with the majority of samples at 30 million reads. Downstream analysis was limited to gene transcripts from autosomes. Transcripts were analyzed to identify differentially expressed genes (DEGs) and specific enhancement of pathways was assessed with gene set enrichment analysis (GSEA). Transcriptional profiles were regressed to measures of HIV persistence (total and integrated HIV DNA and unspliced and multiply spliced RNA) for each sex. Supervised analysis of specific pathways was done as described.

Cohort characteristics

Cohort Characteristics	Men	Women
Age in years, median (IQR)	43 (33-48)	41 (35-48)
CD4 nadir cells/uL, median (IQR)	270 (131-442)	214 (111-317)
CD4 at sampling cells/uL, median (IQR)	646 (544-825)	677 (530-861)
Duration of infection years, median (IQR)	7 (4.0-11.5)	8 (4.8-14.3)
Duration of viral suppression years, median (IQR)	3.3 (2.1-6.7)	2.8 (1.8-4.3)
Max pretreatment viral load, median (IQR)	4.74(4.4-5.4)	4.61(3.8-5.2)
CMV positive, *n* (%)	26 (100)	21 (81)
Active HCV infection, *n* (%)	2 (7.7)	1 (3.8)
IDU, *n* (%)	3 (12)	5 (19)
Race, *n* (%)		
White	9 (35)	8 (31)
Black	7 (27)	6 (23)
Hispanic	4 (15)	4 (15)
Asian	2 (8)	3 (12)
Native American	1 (4)	0
Mixed/Multiracial/Other	3 (11)	5 (19)

Reservoir comparisons

HIV reservoir measure	Female fold effect	Confidence Interval	p value
iHIV DNA	1.39	0.57-3.37	0.47
SCA (HMMCgag) adjusted for: - duration of suppression - treatment interruptions	0.23	0.08-0.72	0.011
CA msHIV RNA	0.16	0.05-0.51	0.002
CA msHIV RNA adjusted for: - CD4 nadir - controller phenotype	0.25	0.09-0.71	0.009

Results

Figure 1: Transcriptome profiles segregate by sex.

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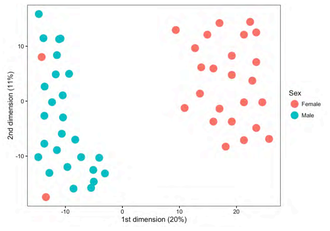

B

LIMMA: t-test		
Comparison	# DEGs p-value < 0.05	# DEGs 5% FDR
Females vs Males	2943	1429
GSEA		
Comparison	# DEGs p-value < 0.05	# DEGs 5% FDR
Females vs Males	23	16

C

NAME	NOM_pval	FDR_qval
HALLMARK_HEME_METABOLISM	0	0.006
HALLMARK_MYC_TARGETS_V1	0	0.0055
HALLMARK_OXIDATIVE_PHOSPHORYLATION	0	0.00533
HALLMARK_PROTEIN_SECRETION	0	0.00701
HALLMARK_INTERFERON_ALPHA_RESPONSE	0	0.00781
HALLMARK_MTORC1_SIGNALING	0	0.00834
HALLMARK_DNA_REPAIR	0	0.0151
HALLMARK_INTERFERON_GAMMA_RESPONSE	0	0.0203
HALLMARK_APOPTOSIS	0	0.0298
HALLMARK_ADIPOGENESIS	0	0.0475
HALLMARK_UV_RESPONSE_UP	0.001	0.0385
HALLMARK_PI3K_AKT_MTOR_SIGNALING	0.002	0.0141
HALLMARK_ANDROGEN_RESPONSE	0.002	0.0312
HALLMARK_UNFOLDED_PROTEIN_RESPONSE	0.004	0.0309
HALLMARK_IL2_STAT5_SIGNALING	0.012	0.0817
HALLMARK_G2M_CHECKPOINT	0.016	0.109
HALLMARK_ALLOGRAFT_REJECTION	0.017	0.123
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY	0.02	0.0248
HALLMARK_HYPOXIA	0.024	0.12
HALLMARK_ESTROGEN_RESPONSE_EARLY	0.029	0.15
HALLMARK_P53_PATHWAY	0.034	0.153
HALLMARK_TGF_BETA_SIGNALING	0.037	0.0446
HALLMARK_TNFA_SIGNALING_VIA_NFKB	0.04	0.151

Figure 1. (A) Multidimensional scaling analysis demonstrates that 20% of the variability in gene expression is attributable to sex. (B) 1429 differentially expressed genes were identified and 16 pathways in gene set enrichment analysis were noted to vary by sex at the 5% false discovery rate cut off. (C) Pathways with differential expression nominal p value of <0.05 are shown, all pathways were upregulated in women relative to men.

Figure 2: A coordinated network of interferon stimulated genes (ISGs) is upregulated in women

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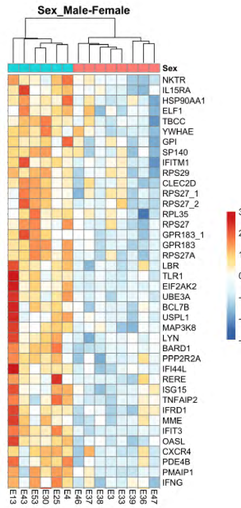

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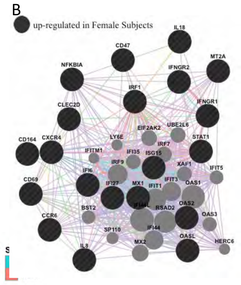

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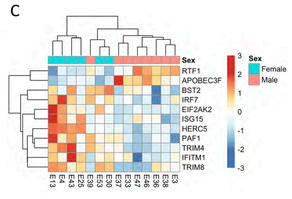


Feature	FDR
response to type I interferon	3.01E-30
cellular response to type I interferon	3.01E-30
type I interferon signaling pathway	3.01E-30
response to virus	7.02E-26
response to interferon-gamma	6.89E-17
regulation of viral genome replication	9.59E-17
negative regulation of viral genome replication	5.33E-16
viral genome replication	1.10E-15
interferon-gamma-mediated signaling pathway	4.63E-15
cellular response to interferon-gamma	4.19E-14

Figure 2. (A) Supervised analysis of the top ISGs differentially expressed by standard deviation in women and men. ISG defined by prior studies[3] (B) GeneMANIA algorithm analysis to identify networks of coordinated gene expression identifies multiple genes and multiple features of antiviral responses upregulated in women. (C) Using an innate restriction factor filter, distinct patterns of gene expression segregate men and women. Restriction factor gene set identified based on prior work relating RF expression to HIV reservoir[4]

Figure 3: Inflammasome genes show differential expression by sex

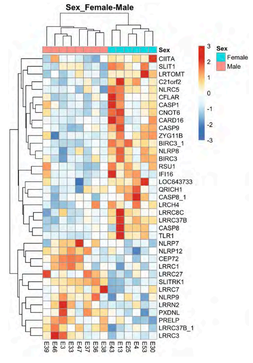


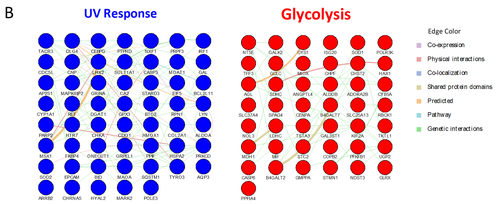
Figure 3. Inflammasome components show distinct regulation in men versus women. Among the genes that are differentially expressed is NLR12, previously reported to have a role in modulating NFkappaB signaling. Genes that are considered part of the inflammasome were extracted from 'inflammasome.domains' which lists the PROSITE protein domains related to the inflammasome and then the bioconductor package 'org.Hs.eg.db' was used to map these to gene symbols or entrez gene ids

Figure 4: Gene pathways associated with reservoir measures are distinct for women and men

A

MEN	WOMEN
HALLMARK_HEME_METABOLISM	HALLMARK_GLYCOLYSIS*
HALLMARK_COMPLEMENT*	HALLMARK_OXIDATIVE_PHOSPHORYLATION*
HALLMARK_UV_RESPONSE_UP*	HALLMARK_MYC_TARGETS_V1*
HALLMARK_KRAS_SIGNALING_DOWN	HALLMARK_MTORC1_SIGNALING
	HALLMARK_G2M_CHECKPOINT
	HALLMARK_E2F_TARGETS*
	HALLMARK_INFLAMMATORY_RESPONSE

B



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Males - CD4 - Reservoir			Females - CD4 - Reservoir		
NAME	NES	NOM_pval	NAME	NES	NOM_pval
HALLMARK_MYC_TARGETS_V1	1.74	0	HALLMARK_FATTY_ACID_METABOLISM	1.9	0
HALLMARK_OXIDATIVE_PHOSPHORYLATION	1.52	0	HALLMARK_MYC_TARGETS_V1	1.6	0
HALLMARK_PROTEIN_SECRETION	1.38	0.0255	HALLMARK_PROTEIN_SECRETION	1.53	0.0063
HALLMARK_TNFA_SIGNALING_VIA_NFKB	1.3	0.039	HALLMARK_MTORC1_SIGNALING	1.45	0.00422
HALLMARK_MYOGENESIS	-1.32	0.0337	HALLMARK_PEROXISOME	1.41	0.0129
HALLMARK_E2F_TARGETS	-1.33	0.0272	HALLMARK_INTERFERON_ALPHA_RESPONSE	1.41	0.0279
HALLMARK_APICAL_SURFACE	-1.38	0.0586	HALLMARK_ADIPOGENESIS	1.31	0.0288
HALLMARK_G2M_CHECKPOINT	-1.4	0.0141	HALLMARK_OXIDATIVE_PHOSPHORYLATION	1.29	0.0402
HALLMARK_APICAL_JUNCTION	-1.58	0	HALLMARK_APOPTOSIS	-1.32	0.0336
HALLMARK_MITOTIC_SPINDLE	-1.62	0	HALLMARK_INFLAMMATORY_RESPONSE	-1.34	0.026
			HALLMARK_MITOTIC_SPINDLE	-1.35	0.0151
			HALLMARK_P53_PATHWAY	-1.38	0.0162
			HALLMARK_MYOGENESIS	-1.45	0.00371
			HALLMARK_ESTROGEN_RESPONSE_EARLY	-1.49	0.00187
			HALLMARK_TNFA_SIGNALING_VIA_NFKB	-2.03	0

Figure 4. (A) Gene pathways positively associated with reservoir measures (combined output of HIV DNA and RNA). * indicates pathways also identified in an independent cohort. (B) Leading edge genes for two of the associated pathways, UV response (DNA damage inducer of senescence) and the glycolysis pathway (associated with features of active metabolism and functional T cell responses. (C) Models integrating reservoir measures and CD4 cell phenotypes identify unique associations in men and women.

Conclusions

In a well-matched cohort of HIV-infected ART-treated, virally suppressed women and men, there is sex specific regulation of gene expression. Women show enrichment of antiviral pathways along and differential expression of components of the inflammasome pathway, including some with regulatory function. Sex-specific analysis of HIV reservoir correlates identifies different gene pathways in men and women. Biological sex determines distinct transcriptional patterns that are related to HIV reservoir, with sex-specific implications for cure strategies

References

[1]Scully, EP Sex Differences in HIV Infection, Curr HIV AIDS Reports, *in press*; [2]Gandhi M et al., Does Patient Sex Affect human immunodeficiency levels?, CID, 2002; [3]Waddell SJ et al., Dissecting Interferon-Induced Transcriptional Programs in Human Peripheral Blood Cells, PLOS One, 2010; [4]Abdel-Mohsen M et al., Select host restriction factors are associated with HIV persistence during antiretroviral therapy, AIDS, 2015

Acknowledgements

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