Host gene expression, cytokine/chemokine profiles and HIV-1 outcomes from pre-infection to two years post-infection

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Objectives

1) To identify patterns of gene expression and chemokine/cytokine changes over the first two years of HIV-1 infection.

2) Determine if the magnitude of those responses associate with clinical outcomes during HIV-1 infection.

3) Evaluate if gene expression early during HIV-1 infection can predict later clinical HIV-1 outcomes.

Background

- Acute HIV-1 infection characterized by ramp-up in HIV-1 replication, decreased CD4+ T cell counts, storm of inflammatory cytokines and an expansion of HIV-1 specific adaptive immune responses (Cohen et al, 2011).
- Immune activation persists and is thought to be a driving force behind eventual CD4+ T-cell loss and development of AIDS (Paiardini and Müller-Trutwin 2013).
- Pathogenic SIV infection of rhesus macaques (RMs) leads to rapid up-regulation of interferon stimulated genes (ISGs) that persists into chronic infection (Bosinger et al. 2009) whereas sooty mangebys (a natural host of SIV) resolve ISG expression.

Methods

- **Participant selection**
  - HIV-1 seroconverters from two African HIV-1 serodiscordant couple cohorts (Celum et al 2010, Lingappa et al. 2012).
  - Whole blood and plasma were collected before and/or after HIV-1 infection.
- **mRNA expression microarrays**
  - Microarrays performed on whole blood using IlluminaHumanV3 BeadChips.
  - Gene expression data was preprocessed using R (www.r-project.org) and Bioconductor (Gentleman et al.) as previously described (Fukazawa et al. 2012; Caskey et al. 2011).
- **Mesoscale cytokine/chemokine assays**
  - Plasma cytokine/chemokine concentrations measured using Mesoscale multiplex assays.
  - Analytes included: CD14, CD40L, IFN-α2a, IFN-β, IFN-g, IL-10, IL-15, IL-1a, IL-1b, IL-6 ,IP-10, TNF-α, TNF-RI and TNF-RII
- **Statistical methods**
  - Linear mixed models (LMM) to determine associations between num. of days since HIV-1 infection and changes in gene expression or analyte conc.
  - Changes in gene expression/analyte levels = difference between pre and post-infection log2 levels.
  - Flexible forms of associations were fit using natural cubic spline and simple linear regression lines. We chose the best-fitting model.
  - Associations between gene expression and analyte concentrations determined using sparse partial least squares regression (Le Cao et al, 2011).
  - Pathway and Network Analysis conducted using Ingenuity Pathway Analysis.
  - Cell type specific expression filters used to identify expression signatures from cell subsets.
  - IFN response genes were filtered to type I (IFN-α) and type II (IFN-γ) responses using previously published lists (Waddell et al. 2010; Caskey et al. 2011)

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Whole blood and plasma collected before and after HIV-1 infection

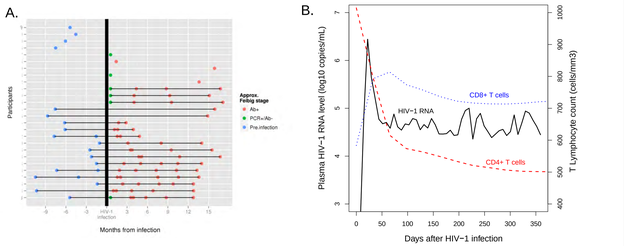
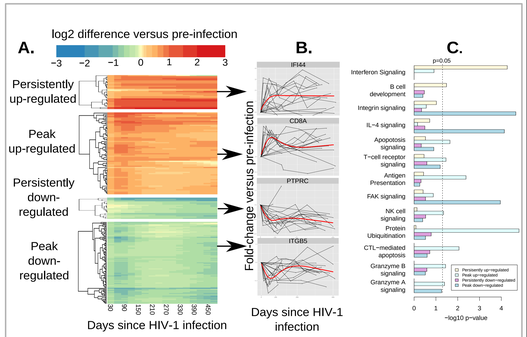


Figure 1: A) Sample collection time points relative to HIV-1 infection for 26 HIV-1 seroconverters included in this study. B) Loess curves for plasma HIV-1 RNA and CD4+ and CD8+ T cells during the first year after HIV-1 acquisition for 150 HIV-1 seroconverters. Baseline CD4+ and CD8+ T cell counts were set to the expected counts for HIV-1 uninfected individuals.

Genes in IFN pathways were >60 times as likely to be persistently up-regulated by >1.5-fold after HIV-1 infection

- Five gene expression trajectories after HIV-1 infection
  1) Persistently up-regulated
  2) Up-regulated followed by declining expression (peak up-regulated).
  3) Persistently down-regulated
  4) Down-regulated followed by increased expression (peak down-regulated).
  5) No change after HIV-1 infection.
- CTL response genes over-represented in the peak up-regulated group.
- IFN-stimulated genes were persistently up-regulated genes.
- IFN-stimulated genes underwent the largest changes in gene expression after infection.



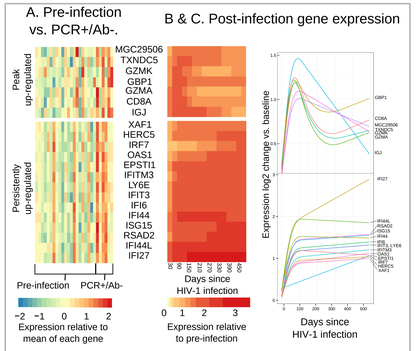


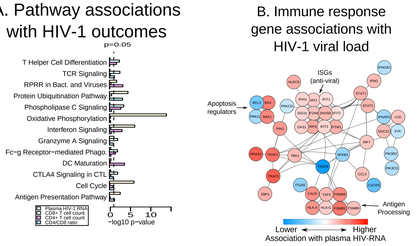
Figure 3: PCR+/Ab- versus pre-infection samples and longitudinal trajectories for 21 genes found to be up-regulated after HIV-1 acquisition by >2-fold (FDR<5%).

Figure 2: A) Heatmaps showing four trajectories of gene expression changes after HIV-1 infection versus pre-infection for 1,346 genes that had FDR<5% for changing after HIV-1 acquisition. B) Representative gene expression trajectories from each trajectory category. C) Ingenuity pathway analysis of gene trajectories.

Gene inclusion	Trajectory		Type I IFN OR (95% CI)	Type I IFN p	Type II IFN OR (95% CI)	Type II IFN p
Genes with p<0.05 & fold-change >1.5	Up	Persistent	83.4 (39, 177)	$1.7x10^{-30}$	63 (31, 126)	$1.16x10^{-31}$
		Peak	5.7 (3.4, 9.4)	$1.2x10^{-11}$	4.8 (2.8, 8.4)	$2.26x10^{-8}$
	Down	Persistent	2.1 (0.5, 8.7)	0.3	2.3 (0.6, 9.8)	0.3
		Peak	2.5 (1.4, 4.5)	0.002	2.8 (1.6, 5)	0.0004

Table 1: Associations between genes in Type I and II Interferon pathways and gene expression trajectory groups. Odds ratios (OR) and 95% confidence intervals (CI) represent the relative odds that genes annotated to the Type I or Type II IFN groups changed following acquisition compared to genes not annotated to that group.

IFN stimulated genes and CXCL10 had the strongest associations with higher HIV-1 RNA in cross-sectional analyses



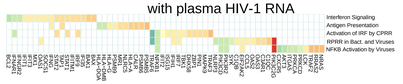




Figure 5: Pathway and network analyses of cross-sectional gene expression associations with HIV-1 clinical outcomes.

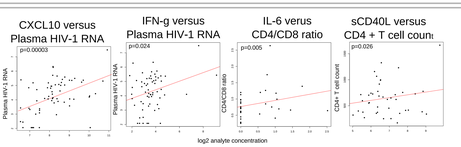


Figure 6: Cross-sectional associations between chemokines/cytokines concentrations and HIV-1 outcomes.

	Type I IFN OR (95% CI)	Type I IFN p-value	Type II IFN OR (95% CI)	Type II IFN p-value
CD4+ T cell count	7.8 (4.4, 13.7)	$9.3x10^{-13}$	4.3 (2.1, 8.8)	$4.5x10^{-5}$
CD8+ T cell count	0.8 (0.1, 5.7)	0.8	0.9 (0.1, 6.4)	0.9
CD4/CD8 ratio	1.6 (0.9, 2.5)	0.06	1.6 (0.9, 2.5)	0.07
Plasma HIV-1 RNA	3.6 (2.7, 5.0)	$4.1x10^{-16}$	4.1 (3.0, 5.6)	$3.7x10^{-18}$

Table 2: Associations between genes in Type I and II Interferon pathways and HIV-1 clinical outcomes. Odds ratios (OR) and 95% confidence intervals (CI) represent the relative odds that genes annotated to the Type I or Type II IFN groups changed following acquisition compared to genes not annotated to that group.

Plasma CXCL10, TNF-α and IFN-γ concentrations were persistently increased after HIV-1 infection and were associated with expression of IFN-stimulated genes

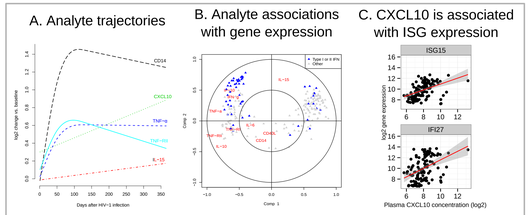
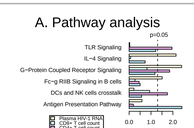
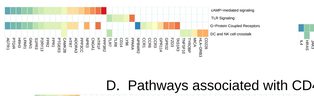


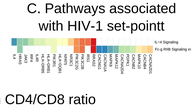
Figure 4: A) Mean trajectories of post-infection plasma inflammatory marker changes versus pre-infection following HIV-1 acquisition. B) Correlation circle plot from MixOmics analysis comparing serum cytokine/chemokine levels to expression values for genes to be up or down-regulated by a factor >2 following HIV-1 infection (FDR<5%). The closeness of points represents the strength of correlation between two variables. C) Associations between serum CXCL10 concentrations and ISG expression.

Early expression of genes in immune response pathways is associated with HIV-1 clinical outcomes 200+ days after infection

- We assessed if gene expression <90 days after infection could predict HIV-1 clinical outcomes >200 days after infection.
- No associations with FDR<5%.
- Pathway analysis found that immune responses pathways were predictive.







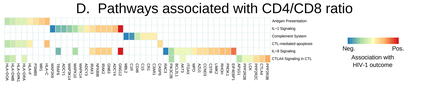


Figure 7: A) Ingenuity pathway analysis of associations between gene expression <100 days after HIV-1 infection and HIV-1 clinical outcomes 200+ days after infection.

Conclusions

- ISGs were more likely to be persistently up-regulated after infection and this could be a factor in chronic immune activation.
- Plasma levels of CXCL10, TNF-α and IFN-γ were persistently increased after HIV-1 infection are were highly correlated with ISG expression.
- Cross-sectionally, ISGs were associated with increased plasma HIV-1 RNA levels and lower CD4+ T cell counts.
- Early expression of ISGs was not predictive of HIV-1 set-point >200 days after infection.
- However, expression of genes in other immune response pathways was predictive of multiple HIV-1 clinical outcomes 200+ days after infection.
- Further work is needed to determine if targeting genes in pathways that predict clinical outcomes could alter disease progression.

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