920

HIV Altered mRNA Expressions of Type 1 IFN Pathway Related to Activation/Exhaustion in CD4+ T Cells

Chi-Lin Lee, PhD[1], Chia-Hao Wang, PhD[1], Erik Serrao, PhD[2,3], Toni Frederick, PhD[1], Kerry Meekin, MPH[1], Patricia Anthony, CLS[1], David Arribas-Layton[1], and Andrea Kovacs, MD[1]

[1]Pediatrics, Keck School of Medicine, University of Southern California, Los Angeles, CA, [2]Medicine, Harvard University, Boston, MA, [3]Pharmacology and Pharmaceutical Sciences, University of Southern California, Los Angeles, CA.

Abstract

Background: Recently, the LCMV model study suggests a mechanism whereby persistent viruses modulate Type I Interferon (IFN)-signaling during chronic infection leading to hyperactivation and immune exhaustion in mice. HIV+ patients treated with peg-IFN-α had sustained virologic suppression and decreased levels of integrated HIV DNA[1]. These findings suggest that type-1 IFN may play a key role in supporting both protective and pathogenic effects in HIV infection. Here we identified 18 host genes that are involved in Type-1 IFN response: IFNs & Receptors, IFN-signaling, and IFN-stimulated genes (ISGs)/cytokines in HIV+ patients and healthy controls. Expression levels of selected genes in association with race, sex, age, infection status, viremic status, and immune activation/exhaustion markers were analyzed in this study.

Methods: CD4+ cells were purified from PBMCs from 49 subjects (33 HIV+ and 16 healthy controls) by using anti-CD4 microbeads (Miltenyi Biotec). mRNA was isolated and reverse transcribed to cDNA by iScriptTMcDNA Synthesis Kit (Bio-Rad) and expression levels were evaluated by RT-PCR array (Applied Biosystems). β-actin and GAPDH were used as housekeeping genes to normalize gene expression results. Using gene expression as the outcome variable in linear regression models, expression levels were first compared by the following groups: age at the time of specimen collection, ethnicity, sex, HIV infection status (adjusting for age) and viremic status among infected subjects. The associations between gene expressions and immune activation/exhaustion markers were modeled adjusting for age and study group among 49 subjects, and adjusting for viremic status among 33 HIV-infected subjects. The immune activation and exhaustion markers were categorized by tertile using the lowest tertile as the reference group. The fold changes were calculated from the $2^{-\Delta\Delta CT}$ method using regression estimates and robust standard errors were used to obtain p-values. To account for the multiple testing issues, the Benjamini and Hochberg method was implemented to control false discovery rate (FDR) at 0.05 levels. Finally, the correlations between interferon genes and immune activation/exhaustion markers within each study group were assessed using spearman correlation tests.

Results: Comparing HIV-infected subjects to healthy controls, gene expression levels of IFNAR1 and JAK1 were significantly down-regulated while IL15, ISG15, MX1 and OAS1 were significantly up-regulated adjusting for age. Expression levels of CXCL10, IFNAR1, JAK1 and OAS1 were significantly associated with HIV viremic status. After adjusting for age and study groups, levels of CD4+ T cell activation (CD38+HLA-DR+) were significantly inversely correlated with IFNA1 and IFNB1. Among HIV-infected subjects, OAS1 was significantly up-regulated comparing the highest to the lowest tertile of CD4+ T cell exhaustion (PD-1+) after adjusting for viremic status. In comparison, IFNA1 was significantly down-regulated and CXCL10, IRF7 and OAS1 were significantly up-regulated comparing the highest to the lowest tertile of CD38+HLA-DR+. IFNB1 and IFNAR1 expressions levels were natively correlated with CD4+CD38+DR+ among HIV-infected non-viremic and untreated subjects, respectively. Among HIV-infected high viral load subjects, JAK1 expression level was negatively correlated with CD4+PD1+ marker.

Conclusion: Our results demonstrate that during HIV infection there is a complex, defective type-1 IFN response, including decreased IFN-α receptors/signaling genes and increased ISGs and the inflammatory cytokine gene at the same time. These results suggest that HIV could perpetuate interferon-related immune dysfunction and activation that underlies HIV pathogenesis, and thus reveals possible molecular mechanisms contributing to T cell exhaustion.

Background

Two recent studies using the LCMV model suggest a mechanism whereby persistent viruses modulate both Type I IFN-signaling leading to hyperactivation and negative immune regulatory molecules leading to immune exhaustion and dysregulation. Blocking type-1 IFN receptors decreased chronic inflammatory signaling, proinflammatory cytokines, and immune suppressive/regulatory components (PD-1, IL-10) during LCMV persistent infection in mice. In acute infection this resulted in viral persistence. Plasma IFN levels have also been shown to correlate with immune activation. However, a recent proof-of-concept study demonstrated that HIV+ patients treated with pegylated IFN-alfa-2a (peg-IFN-α) had sustained virologic suppression and decreased levels of integrated HIV DNA. Interferon has anti-HIV properties with 0.5 log reduction in plasma RNA. These findings suggest that type-1 IFN may play a key role in supporting both protective and pathogenic effects in HIV infection. Moreover, type 1 IFN induction also plays a critical role in CD4+ T cell depletion during HIV infection[2]. Here we identified 18 host genes that are involved in Type-1 IFN response: IFNs & Receptors (IFNAR1, IFNA1, IFNB1), IFN-signaling (STAT1, STAT2, JAK1, IRF9, IRF7), and IFN-stimulated genes (ISGs)/cytokines (CD80, IL-15, ISG15, MX1, OAS1, OAS2, TRIM22, TRIM5, CXCL10, IFIH1) in CD4+ T cells in HIV+ patients and healthy controls. Expression levels of selected genes in association with race, sex, age, infection status, viremic status, and immune activation/exhaustion markers were analyzed in this study.

Methods

Study Design

- Subjects were selected from a Natural History, Long-term Follow-up and Pathogenesis Study of HIV Infection at the Maternal, Child, Adolescent (MCA) Center for Infectious Diseases and Virology at the Los Angeles County + University of Southern California Medical Center (LAC+USC).
- Informed consent was obtained for each patient in this IRB-approved study.
- At routine clinic visits, blood specimens were stored in the MCA repository for future research studies.
- Additional specimens from uninfected controls were provided by volunteers for an IRB approved sub-study of the Natural History Study.

Subject Selected

- 33 HIV- infected patients
  - → 9 were untreated with a viral load >50 copies/ml: Untreated/viremic
  - → 15 had antiretroviral treatment and a viral load <50 copies/ml for 2 years: Treated/non-viremic
  - → 9 had treatment and a viral load >10,000 copies/ml: Treated/high viral load
- 16 HIV uninfected controls

Table 1: Partcipant Demographics

Characterstics	HIV-infected, Untreated, Detectable	HIV-infected, ART-Treated, High Viral load	HIV-infected, ART-Treated, Non-Viremic	Uninfected
Number participants	9	9	15	16
Median age, years (range)	30 (17-55)	24 (13-40)	35 (17-43)	14 (11-34)
Sex, % female	56	78	80	69
Ethnicity, % Hispanic	56	78	93	88
Median CD4+ count (range)	541 (338-942)	299 (23-713)	774 (405-976)	907* (733-1812)
Median CD4% (range)	23 (6.8-42)	21.1 (1.1-29.8)	32.6 (18-45.9)	43* (33.3-53.8)
Median Viral RNA copies (range)	2,505 (129-1,880,000)	73,258 (11,776-172,136)	<50	-
Number perinatally infected	0	5	3	-
Number behaviorally infected	9	4	12	-

* Among 12 patients with CD4 count and percentage data.

Mean age was significantly lower for controls compared to HIV-infected patients. A smaller percentage of untreated/viremics were female and Hispanic compared to other HIV-infected groups. Those with high viral load had the lowest CD4 numbers. Viral loads for the untreated/viremics ranged from 129 - 1,880,000 copies/ml.

Laboratory Methods

- CD4+ T cells were purified from banked PBMCs by using anti-CD4 microbeads (Miltenyi Biotec).
- mRNA was isolated and reverse transcribed to cDNA by iScriptTMcDNA Synthesis Kit (Bio-Rad) and expression levels were evaluated by RT-PCR array (Applied Biosystems).
- PCR array was purchased and assembled by Life Technologies and experimental results analyzed using the OpenArray® Real-Time PCR System at the USC School of Pharmacy Translational Research Laboratory (USC TRLab).
- Before loading to PCR Arrays, concentration of all the cDNA samples were increased by TaqMan® PreAmp Master Mix pre-amplification Kit (Applied Biosystems) according to the manufacturer's protocol.
- β-actin and GAPDH were used as housekeeping genes to normalize gene expression results.
- 6-color flow cytometry was used to assess expression of HLA-DR, CD38, and PD-1 on CD4+ T cells as a measure of activation and exhaustion using the following fluorochrome-labeled antibodies: CD3-PerCP-Cy5.5, CD4-FITC, HLA-DR-PE-Cy7, CD38-PE, and PD-1-PE (BD Biosciences).
- Analysis was by FACSCanto flow cytometer (BD Biosciences) with FlowJo version 9.4 software (Tree Star, Inc). Compensation was performed with BD CompBeads stained separately with individual mAbs. Gating strategy and background levels of staining were determined by utilizing Fluorescence Minus One (FMO) with isotype controls for all antibodies.

Statistical Methods

- Interferon gene expression levels were measured in Cycles to Threshold (Ct: the cycle number at which logarithmic PCR plots cross a calculated threshold line) and used to determine ΔCt values (ΔCt = Ct of the target gene minus Ct of the housekeeping gene).
- Association analyses were modeled by linear regressions using ΔCt as the outcome variable.
  a. Regression estimates were used to calculate fold changes by the $2^{-\Delta\Delta CT}$ method and robust standard errors were used to obtain p-values.
  b. P-values were screened using the Benjamini and Hochberg approach to control FDR at the 0.05 level.
  c. Immune activation and exhaustion markers were categorized by tertiles using the lowest tertile as the reference group.
- Within each study group, immune activation and exhaustion markers were treated as continuous variables and their relationships with interferon genes were reported by spearman correlations and p-values.

Results

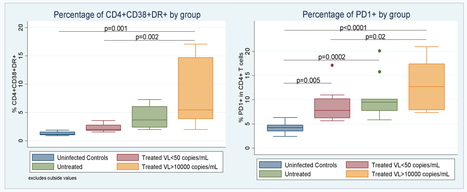
Table 2: Interferon Gene Expression Levels (ΔCt) by Study Group

Gene	Mean ΔCt (range)				P-value
	HIV-infected, Untreated, Detectable	HIV-infected, ART-Treated, High Viral load	HIV-infected, ART-Treated, Non-Viremic	Uninfected	
cd80	11.31 (10.29, 13.09)	11.61 (10.97, 12.4)	11.34 (10.7, 12.61)	11.95 (10.18, 12.79)	0.1044
cxcl10	9.62 (6.5, 12.87)	9.13 (7.23, 10.81)	11 (7.56, 13.48)	11.56 (6.64, 13.59)	0.0007
ifih1	7.73 (6.66, 8.83)	7.69 (6.38, 9.48)	7.8 (7.38, 8.39)	7.5 (6.92, 8.55)	0.2058
ifna1	13 (9.91, 15.08)	14.06 (13.02, 15.01)	11.68 (8, 13.94)	13.48 (11.45, 14.58)	0.1441
ifnar1	6.42 (5.93, 7.29)	6.6 (5.86, 7.42)	6 (5.55, 6.45)	5.62 (5.2, 6.16)	<0.0001
ifnb1	12.71 (10.17, 13.59)	13.29 (12.13, 14.23)	11.93 (8, 14.2)	13.01 (11.02, 14.03)	0.1042
il15	7.96 (7.29, 8.34)	7.9 (7.2, 8.53)	8.18 (7.27, 9.29)	8.77 (7.66, 9.76)	0.0009
irf7	4.23 (3.3, 5.32)	3.21 (2.34, 3.86)	4.28 (3.3, 6.06)	4.22 (3.42, 5.03)	0.0001
irf9	6.14 (5.39, 7.18)	6 (5.2, 6.79)	5.88 (5.38, 6.49)	5.83 (5.4, 6.33)	0.561
isg15	4.4 (3.36, 6.5)	3.01 (1.95, 4.87)	4.31 (3.69, 5.05)	4.4 (3.74, 5.09)	0.001
jak1	2.07 (1.32, 3.37)	2.43 (1.13, 3.94)	1.4 (1.01, 2.07)	1.26 (0.59, 1.92)	0.0004
mx1	4.47 (2.89, 5.31)	3.49 (1.94, 4.81)	4.59 (4.09, 5.35)	4.81 (3.78, 5.32)	0.0011
oas1	6.95 (5.2, 8.87)	5.52 (4.08, 6.74)	7.28 (6.2, 8.65)	7.43 (5.93, 9.64)	0.0001
oas2	4.59 (3.01, 6.44)	3.57 (2.32, 4.96)	4.63 (3.79, 6.12)	4.16 (3.29, 6.24)	0.023
stat1	2.7 (1.56, 4.6)	2.58 (1.61, 4.47)	2.99 (2.09, 4.34)	2.72 (1.43, 3.92)	0.601
stat2	5.21 (4.23, 6.73)	4.64 (3.79, 5.58)	5.12 (4.54, 6.4)	4.91 (4.18, 6.38)	0.2492
trim22	2.86 (1.89, 4.14)	2.62 (1.98, 3.75)	2.63 (2.14, 3.44)	2.6 (2.04, 3.19)	0.8364
trim5	7.62 (6.44, 10.13)	7.11 (5.96, 8.94)	7.2 (6.3, 9.53)	6.69 (5.73, 9.69)	0.3366

Note: 1. Robust standard errors from linear regressions were used to calculate p values.
2. The Benjamini and Hochberg method was used to account for multiple comparisons. Controlling FDR at 0.05 level, the significant p-values are highlighted in red color.

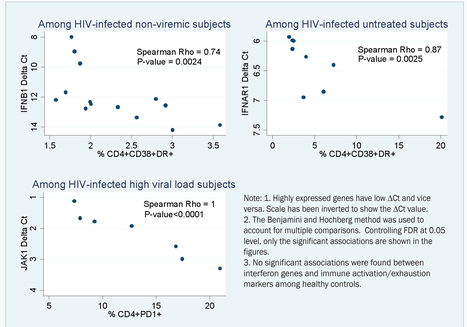
- The expression levels were significantly different between study groups for the following genes: CXCL10, IFNAR1, IL15, IRF7, ISG15, JAK1, MX1, OAS1, and OAS2.

Figure 1. Levels of CD4+CD38+DR+ and PD1+ by Study Group



- Percentages of CD4+CD38+DR+ T cells were significantly lower in healthy controls and HIV-infected treated/non-viremic patients compared to treated/high viral load patients.
- Percentages of CD4+PD1+ T cells were significantly lower in healthy controls compared to all groups of HIV-infected patients. CD4+PD1+ T cell percentages were significantly lower in treated/non-viremic patients than treated/high viral load patients.

Figure 3. Relationship Between Interferon Genes and Immune Activation/Exhaustion Markers within each of the Study Groups



Note: 1. Highly expressed genes have low ΔCt and vice versa. Scale has been inverted to show the ΔCt value.
2. The Benjamini and Hochberg method was used to account for multiple comparisons. Controlling FDR at 0.05 level, only the significant associations are shown in the figures.
3. No significant associations were found between interferon genes and immune activation/exhaustion markers among healthy controls.

- Among infected non-viremic: IFNB1 was negatively associated with CD4+CD38+DR+.
- Among infected untreated: IFNAR1 was negatively associated with CD4+CD38+DR+.
- Among infected high viral load: JAK1 was negatively associated with CD4+PD1+.

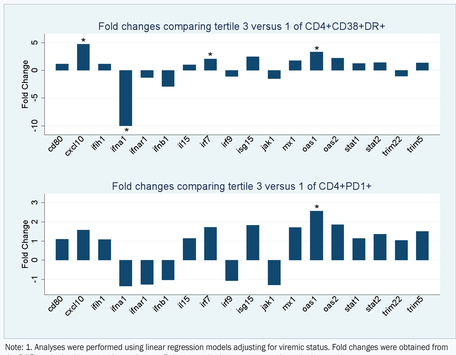
Table 3: Analysis of Gene Expression in Relation to Demographic and Clinical Characteristics

Gene	Race (9 Non-Hispanic vs. 40 Hispanic (Ref))		Sex (14 male vs 35 female (Ref))		Age (25 subjects >=23 vs 24 subjects <23(Ref))		Infection Status (33 infected vs. 16 uninfected (Ref))		Among infected: viremic status (18 viremic vs. 15 non-viremic (Ref))	
	Fold Change	p-value	Fold Change	p-value	Fold Change	p-value	Fold Change	p-value	Fold Change	p-value
cd80	1.13	0.6	-1.17	0.36	1.39	0.03	1.25	0.28	-1.09	0.62
cxcl10	2.25	0.1	-1.72	0.26	1.72	0.16	3.14	0.02	3.1	0.006
ifih1	1.29	0.09	1.13	0.26	-1.12	0.32	-1.16	0.32	1.07	0.65
ifna1	-2.4	0.09	-2.15	0.16	3.16	0.05	1.14	0.85	-3.59	0.1
ifnar1	-1.03	0.82	-1	0.99	-1.21	0.08	-1.71	0.0001	-1.42	0.001
ifnb1	-1.2	0.42	-1.14	0.59	1.47	0.14	1.23	0.45	-2.09	0.06
il15	1.03	0.77	-1.22	0.13	1.63	0.00002	1.44	0.01	1.19	0.14
irf7	1.11	0.57	-1.06	0.73	1.07	0.63	1.33	0.11	1.47	0.04
irf9	1.08	0.46	1	0.96	-1.04	0.69	-1.16	0.19	-1.14	0.26
isg15	-1.06	0.84	-1.19	0.44	1.08	0.63	1.74	0.008	1.52	0.06
jak1	-1.01	0.96	1.02	0.89	-1.24	0.14	-1.69	0.005	-1.81	0.0003
mx1	1.49	0.09	1.07	0.69	1.13	0.43	1.81	0.0003	1.53	0.02
oas1	1.46	0.28	1.08	0.77	1.1	0.69	2.18	0.003	2.06	0.007
oas2	1.23	0.45	1.13	0.55	-1.31	0.14	1.07	0.76	1.46	0.1
stat1	1.39	0.13	1.12	0.55	-1.1	0.57	-1.04	0.86	1.27	0.25
stat2	1.08	0.68	1.08	0.6	-1.08	0.58	-1.03	0.86	1.15	0.43
trim22	1.15	0.23	1.1	0.29	-1.03	0.77	-1.07	0.58	-1.08	0.56
trim5	1.1	0.72	1.28	0.26	-1.41	0.13	-1.5	0.13	-1.11	0.7

Note: 1. Analyses were performed using linear regression models. Fold changes were obtained from the $2^{-\Delta\Delta CT}$ method using regression estimates. Robust standard errors were used to calculate p-values. Univariate analyses were performed for race, sex, age and viremic status (among HIV-infected subjects). The association with infection status was modeled adjusting for age since uninfected subjects tend to be younger. Age was not adjusted in other models for race, sex and viremic status since it was not a confounder.
2. Ref: Reference group.
3. The Benjamini and Hochberg method was used to account for multiple comaprisons. Controlling FDR at 0.05 level, the significant p-values are highlighted in red color.

- Gene expressions were not significantly associated with race and sex.
- IL15 was up-regulated 1.63-fold for older subjects (>=23 yr) compared to younger subjects (<23 yr).
- Comparison of HIV-infected vs. healthy controls after adjusting for age showed that IL15, ISG15, MX1 and OAS1 were up-regulated (1.44, 1.74, 1.81, and 2.18-fold respectively) while IFNAR1 and JAK1 were down-regulated (1.71 and 1.69-fold respectively).
- Among the HIV-infected, IFNAR1 and JAK1 were down-regulated for viremic vs. non-viremic patients (1.42 and 1.81-fold respectively) while CXCL10 and OAS1 were up-regulated (3.1 and 2.06-fold respectively).

Figure 2. Fold Changes Comparing the Highest to the Lowest Tertiles of CD4+CD38+DR+ and CD4+PD1+ Among the HIV-infected



Note: 1. Analyses were performed using linear regression models adjusting for viremic status. Fold changes were obtained from the $2^{-\Delta\Delta CT}$ method using regression estimates. Robust standard errors were used to calculate p-values.
2. For CD4+CD38+DR+: Tertile 1 (Ref): CD4+CD38+DR+ <2.3, Tertile 2: 2.3<=CD4+CD38+DR+ <3.7, Tertile 3: CD4+CD38+DR+ >=3.7
For CD4+PD1+: Tertile 1 (Ref): CD4+PD1+<7.5, Tertile 2: 7.5<=CD4+PD1+<10.2, Tertile 3: CD4+PD1+>=10.2. There is no significant difference in gene expression comparing Tertile 2 to 1 for both CD4+CD38+DR+ and CD4+PD1+ markers (graph not shown).
3. The Benjamini and Hochberg method was used to account for multiple comparisons. Controlling FDR at 0.05 level, significant associations are marked by *.

- Among the HIV-infected, CXCL10, IRF7, and OAS1 were significantly up-regulated and IFNA1 was down-regulated comparing the highest to lowest tertile of CD4+CD38+DR+ adjusting for viremic status.
- Only OAS1 was significantly up-regulated comparing those in the highest to lowest tertile of CD4+PD1+.

Table 4: Analysis of Gene Expression in Relation to CD4+CD38+DR+ and CD4+PD1+ Markers Adjusting for Age and Study Group Using 49 Subjects

Gene	CD4+CD38+DR+ marker				CD4+PD1+ marker			
	Fold Change (tertile 2 vs. 1)	P-value	Fold Change (tertile 3 vs. 1)	P-value	Fold Change (tertile 2 vs. 1)	P-value	Fold Change (tertile 3 vs. 1)	P-value
cd80	1.24	0.39	1.32	0.3	-1.13	0.77	1.1	0.81
cxcl10	-2.37	0.21	1.16	0.84	1.08	0.86	1.82	0.2
ifih1	1.01	0.94	-1.16	0.39	-1.1	0.58	-1.26	0.14
ifna1	-12.71	0.002	-22.42	0.007	-	-	-1.67	0.57
ifnar1	1.02	0.88	-1.25	0.14	-1.1	0.53	-1.38	0.06
ifnb1	-6.45	0.002	-9.7	0.001	-1.17	0.71	-1.66	0.26
il15	-1.2	0.27	-1.14	0.47	1.12	0.48	1.4	0.1
irf7	1.14	0.54	1.25	0.3	-1.51	0.16	-1.18	0.44
irf9	1.07	0.63	-1.25	0.21	1.09	0.58	-1.14	0.37
isg15	1.22	0.14	1.53	0.05	-1.08	0.79	1.24	0.53
jak1	1.12	0.38	-1.45	0.08	1.03	0.86	-1.37	0.09
mx1	1.36	0.02	1.32	0.18	-1.08	0.73	1.16	0.41
oas1	1.19	0.47	1.49	0.21	-1.48	0.15	1	0.99
oas2	1.06	0.85	1.07	0.83	-1.44	0.28	-1.32	0.36
stat1	-1.12	0.69	-1.3	0.44	-1.05	0.91	-1.24	0.63
stat2	1	0.99	-1.12	0.7	-1.23	0.4	-1.27	0.26
trim22	1.15	0.45	-1.21	0.38	1.01	0.92	-1.21	0.2
trim5	1.08	0.86	-1.25	0.67	-1.37	0.37	-1.5	0.18

Note: 1. For CD4+CD38+DR+: Tertile 1 (Ref): CD4+CD38+DR+ <1.9, Tertile 2: 1.9<=CD4+CD38+DR+ <3, Tertile 3: CD4+CD38+DR+ >=3
For CD4+PD1+: Tertile 1 (Ref): CD4+PD1+<5.8, Tertile 2: 5.8<=CD4+PD1+<8.5, Tertile 3: CD4+PD1+>=8.5
2. Analyses were performed using linear regression models adjusting for age and group. Fold changes were obtained from the $2^{-\Delta\Delta CT}$ method using regression estimates. Robust standard errors were used to calculate p-values.
3. The Benjamini and Hochberg method was used to account for multiple comparisons. Controlling FDR at 0.05 level, the significant p-values are highlighted in red color.

- After adjusting for age and study group, IFNA1 and IFNB1 expressions were both down-regulated by 22.42-fold (p=0.007) and 9.7-fold (p=0.001) respectively comparing the highest to lowest tertile of CD4+CD38+DR+.
- No significant associations were found between CD4+PD1+ marker and interferon genes.

Summary

▶ Comparing HIV-infected subjects to healthy controls, expression levels of IFNAR1 and JAK1 significantly decreased while IL15, ISG15, MX1 and OAS1 significantly increased. CXCL10, IFNAR1, JAK1 and OAS1 were significantly associated with viremic status among HIV-infected subjects.
▶ Higher levels of CD4+CD38+DR+ marker were strongly associated with down-regulation of IFNA1 and IFNB1.
▶ Among HIV-infected subjects, CXCL10, IRF7, OAS1 were significantly up-regulated while IFNA1 was down-regulated comparing the highest to the lowest tertile of CD4+CD38+DR+ marker. In comparison, only OAS1 was significant in relation to CD4+PD1+ marker.
▶ IFNB1 and IFNAR1 were highly correlated with CD4+CD38+DR+ marker among HIV-infected non-viremic and untreated subjects, respectively. JAK1 was significantly correlated with CD4+PD1+ marker among high viral load patients.

Conclusions

▶ Our results demonstrate that during HIV infection there is a complex, defective type-1 IFN response, including decreased IFN-α receptors/signaling genes and increased ISGs and the inflammatory cytokine gene at the same time. Together, our studies indicate that HIV-1 infection can modify type-1 interferon responsiveness, and alters the expression of IFN-stimulated genes (ISGs) and inflammatory cytokines. These results suggest that HIV could perpetuate interferon-related immune dysfunction and immune activation that underlies HIV pathogenesis, and thus reveals possible molecular mechanisms contributing to T cell exhaustion.

References

1. Azzoni et al., Pegylated interferon alfa-2a monotherapy results in suppression of hiv type 1 replication and decreased cell-associated hiv DNA integration. Journal of Infectious Diseases, 2013;207:213-222.
2. Sivaraman, V., L. Zhang, L. Su, Type I interferon contributes to CD4+ T cell depletion induced by infection with HIV-1 in the human thymus. J. Virol. 85: 9243–9246.
3. Livak, K. J., and T. D. Schmittgen.(2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2- Ct method. Methods 25:402-8.
4. Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B 57, 289–300.

Acknowledgements

This work was supported by funds from NIH/NIAID grants (R01 AI052065, R21 AI081610), a research grant from the Campbell Foundation, and a TL1 Training Grant from the Southern California Clinical Translational Science Institute (SC-CTSI) from the Neamati lab, as well as support from the Maternal, Child, Adolescent Center for Infectious Diseases and Virology.