Potential Role of IL-10 and IL-1 Pathways in IRIS Pathogenesis

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Background and Study Rationale

Inflammation and immune activation play a central role in HIV pathogenesis. An exuberant inflammatory response, termed Immune Reconstitution Inflammatory Syndrome (IRIS), occurs in a subset of patients shortly after starting antiretroviral therapy (ART), and is more common in those with severe lymphopenia and underlying opportunistic infections. If the IRIS response is due to a previously undiagnosed pathogen, it is termed unmasking IRIS. Exacerbation of symptoms from a previously known or treated disease is called paradoxical IRIS. The pathogenesis and etiology of IRIS remain unclear. Pro-inflammatory biomarkers, such as IL-1, IL-6, IL-18 and CRP, have been shown to be differentially expressed in HIV+ patients who develop IRIS. Furthermore, immunoregulatory markers, such as IL-10, have also been shown to be increased in IRIS. The underlying molecular pathways involved in this differential expression are not fully understood. Gene expression analysis by microarray of patient samples prior to ART initiation could allow the unbiased study of genes and molecular signatures in patients before they develop clinical manifestations of IRIS; helping us understand the molecular pathways triggering the syndrome.

Study Design & Methods

- Gene expression profiles measured by microarray analysis of peripheral blood mononuclear cells (PBMC) obtained pre-ART from 140 HIV+ patients. Data initially analyzed by the non-supervised method using Principal Component Analysis (PCA), followed by supervised methods using Ingenuity Pathway Analysis (IPA).
- To detect which molecular pathways might be over-represented in patients that would later develop IRIS we focused on biological function terms as well as upstream regulators that showed statistical significance in IPA. A heat map of genes associated with those pathways was created using JMP. Molecular pathways of interest were created using IPA. The relationship between these genes and the development of IRIS were analyzed using Wilcoxon analysis. The Bonferroni method was used to adjust for multiple comparisons.
- Additionally, our group looked at genes known to be associated with IRIS in literature. We compared these genes in each group using Wilcoxon analysis, and the Bonferroni method was used to adjust for multiple comparisons.

Results

Table 1: Characteristics of Study Participants at baseline (pre-ART) (Median values reported with 25th and 75th percentile).

	Total	IRIS	Non-IRIS	p-value*
Age	38 (30, 45)	35 (29, 42)	38 (31, 46)	0.25
Gender	78% Male	77% Male	78% Male	
CD4 T cells/μL	29 (11, 51)	22 (5, 49)	29 (12, 56)	0.2
CD4 %	3 (2, 7)	3 (1, 6)	3 (2, 7)	0.86
HIV-RNA (log_{10} c/mL)	5.06 (4.72, 5.46)	5.29 (4.86, 5.70)	5.04 (4.53, 5.41)	0.02
Median Days to IRIS	28 (14, 56)			
Type of IRIS	43% Paradoxical, 54% Unmasking			

*Wilcoxon Test

Results

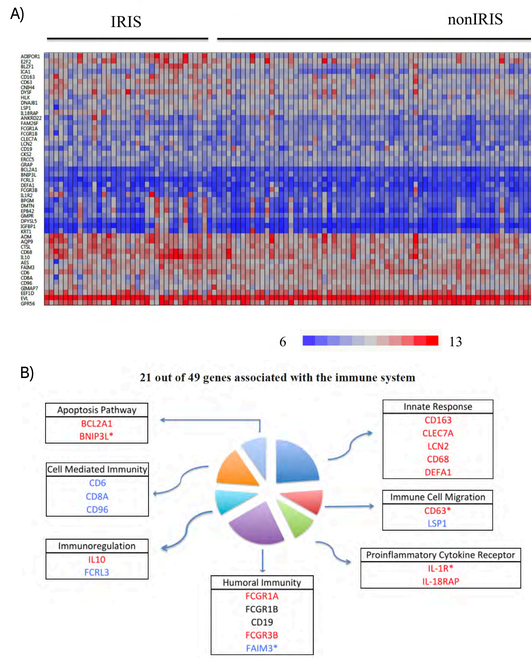


Figure 1.
A) Heat map of genes found to be of interest using IPA analysis. B) Of those genes, we looked at those known to be associated with the immune system. Genes highlighted in red were found to have a significantly higher and those in blue a significantly lower signal in IRIS using Wilcoxon analysis. Genes with an asterisk remained significant using the Bonferroni method.

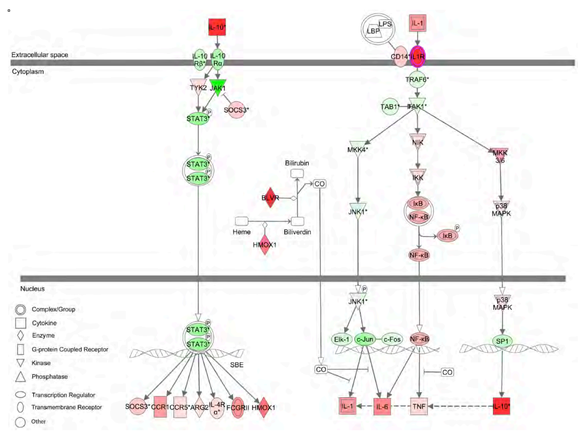


Figure 2. Differential gene expression for transcripts canonical pathway "IL-10 Signaling". Literature curated interactions between gene products are portrayed by shapes indicating type of gene product, while colors indicate the magnitude of difference in mRNA expression observed in this study. Transcripts in red were more abundant in IRIS patients, in green were more abundant in non-IRIS.

Inflammatory Biomarkers	Gene ID	Inflammatory Biomarkers	Gene ID
IL-1B	IL1R1	IL-10	TGIF1
	IL1B		TGIF2LX
	IL1R2*		IL10 ↑
	IL1RAP ↑	IL-17	IL17B
	IL1RL1 ↑		IL17D
IL-6	HGF		IL17RD
	HSF1		IL17RA
	HSF4		IL17RC
	IL6		IL17A
TNFa	TNFAIP1		IL17RE
	TNFAIP2		IL17RB
	TNFAIP6		IL17REL
	TNFAIP3	IL-7	IL7R* ↓
	TNFAIP8 ↓		IL7
	TNFAIP8L2	IL-18	IL18BP
	TNFAIP8L3		IL18R1
	TNF		IL18
CRP	CRP		IL18RAP ↑

Coagulopathic Biomarkers	Gene ID
Tissue Factor	F3
Thrombin	F2R ↓
	F2RL2 ↓
Coagulation Factor VIII	F8A1
Coagulation Factor XI	F11R* ↑
Coagulation Factor XII	F12 ↑

Table 2.
Review of inflammatory and coagulation biomarkers by IRIS status. Genes in bold were found to be significant using Wilcoxon analysis. Genes with an asterisk remained significant when p-values were adjusted for multiple testing. Arrows indicate whether expression was increased or decreased.

From a completed longitudinal cohort of ART-naive patients with CD4 count <100 cells/ul starting ART, PBMCs were available from 140 patients at a pre-ART timepoint. Patients had a median age of 38 (IQR 30, 45) years, CD4 count of 29 (IQR 11, 51) cells/ul, and plasma HIV VL 5.06 (4.72, 5.46) log_{10} copies/mL. Thirty-five patients (25%) developed IRIS, based on ACTG criteria at a median of 28 days after ART initiation.

As seen in Figure 1, Expression signatures do not easily discriminate IRIS versus non-IRIS patients, suggesting that any changes at the molecular level were subtle. The most striking biological theme suggested by IPA analysis was expression of gene subsets associated with inflammatory responses and apoptosis/necrosis. IPA analysis specifically highlighted the IL-1 and IL-10 molecular pathways as shown in Figure 2.

We also reviewed biomarkers known to be associated with IRIS in literature, as well as other known markers of inflammation and coagulation. Using Wilcoxon analysis, we found that genes associated with IL-1, TNF-alpha, IL-10, IL-17, IL-7, IL-18, and thrombin were significantly different in IRIS versus non-IRIS patients. When adjusting with the Bonferroni method, genes associated with IL-1 and IL-7 remained significant.

Conclusion

- This study highlights possible molecular pathways, including IL-1, IL-10, and IL-18, to be involved in IRIS pathology and may have implications for IRIS prevention and treatment.
- Our data suggest a complex interplay between inflammatory and immunoregulatory pathways as well as coagulation factors occurring prior to the IRIS event itself.
- Further analysis is required to gain a better understanding of the connections and associations between the various gene expressions and the molecular pathways potentially involved in the development of IRIS in HIV+ patients that may also have implications in other settings of reversal of immune suppression.

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This research was made possible by Intramural Research Program of the National Institute of Allergy and Infectious Diseases at the National Institutes of Health (NIH)