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Inflammasomes Play a Major Role in TB IRIS Pathogenesis

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Background:

- Tuberculosis (TB) immune reconstitution inflammatory syndrome (IRIS) affects 20% of patients with TB and HIV after antiretroviral therapy (ART) initiation.
- It is associated with significant morbidity and its pathogenesis has still been not fully elucidated.
- We identified differentially expressed RNA transcripts between TB/HIV co-infected patients with and without IRIS.

Methods:

- Subjects in this cohort study (NCT01611402) were TB/HIV co-infected and had been on TB treatment and ART.
- Blood was collected in PAXgene tubes at the time of TB-IRIS or, for those without IRIS, at 8-12 weeks after ART initiation, then cryopreserved.
- RNA was extracted and hybridized on the Affymetrix GeneChip® Human Gene 2.0 ST array. The chip was then stained and scanned.
- Expression of transcripts was compared using ANOVA with multiple test correction using Benjamini–Hochberg false discovery rate method.
- Ingenuity Pathway Analysis (IPA) was used to identify canonical pathways and upstream regulators.

Results:

- Patients with (n=6), and without (n=10) TB-IRIS were enrolled.
- Patient characteristics are listed in Table 1.
- Patients with TB-IRIS had higher plasma HIV RNA (p=0.014), C-reactive protein (CRP, p=0.002) and absolute neutrophil count (ANC, p<0.001) and lower hemoglobin (Hb, p<0.001).

Results Cont.:

Table 1. Patient Characteristics

	IRIS	No IRIS	P Value
Number of patients	6	10	
Male, n (%)	2 (33.3%)	6 (60%)	0.30
Age, median (IQR)	35.5 (32.3-43.8)	37.5 (35.8-43.5)	0.44
Black race, n (%)	5 (83.3%)	6 (60%)	0.59
Days on TB therapy, median (IQR)	50.5 (41.5-65.75)	127.5 (88-159.5)	**0.013**
Days on ART, median (IQR)	12.0 (6.8-23.3)	60.5 (56-69.8)	**0.001**
Days from start of TB Therapy to ART, median (IQR)	37 (31.8-49.3)	45.5 (30.5-103)	0.48
CD4 T-cell count at study entry, cells/μL, median (IQR)	138 (65.8-228)	107 (60.3-238)	0.78
HIV RNA at study entry, $\log_{10}$ copies/mL, median (IQR)	3.4 (3.0-4.0)	1.7 (1.6-2.0)	**0.014**
CRP, mg/dL, median (IQR)	71.5 (39.4-169.5)	1.6 (1.3-6.7)	**0.002**
Hb, g/dL, median (IQR)	9.0 (8.5-9.4)	12.5 (11.5-13.4)	**<0.001**
ANC, x 10^3/μL, median (IQR)	4.1 (3.2-9.4)	1.8 (1.2-2.6)	**<0.001**

- Using p<0.05 as cut off (Benjamini-Hochberg false discovery rate method for multiple test correction), patients with IRIS had 483 differentially expressed transcripts, of which 218 had ≥ ±2-fold difference (193 up and 25 down regulated), when compared to patients with no IRIS.

Table 2. Top Over Represented Pathways Identified by IPA

Top Over Represented Pathways	P Value	Overlap
Inflammasomes	8.13 e-6	4/21 (19%)
TREM-1	7.82 e-5	5/70 (7.1%)
Toll Like Receptor signaling	9.57 e-5	5/73 (6.8%)

Table 3. Potential Upstream Regulators Predicted by IPA

Potential Upstream Regulators	Predicted State	Z Score	P Value
Transglutaminase 2	Activated	3.5	4.22 e-9
Tumour Necrosis Factor	Activated	2.6	1.92 e-2
Interferon-γ	Activated	2.3	4.49 e-4
Interferon-α	Activated	2.2	1.79 e-3
IL-10	Activated	2.2	6.58 e-5
Interferon Regulatory Factor-1	Activated	2.0	1.22 e-4
Triggering Receptor Expressed On Myeloid Cells (TREM)-1	Inhibited	-2.0	2.02 e-2

Results Cont.:

Activation of these pathways and potential upstream regulators would lead to an increase in Caspase-1 breakdown of pro-interleukin (IL)-1b to IL-1b, resulting in a proinflammatory response.

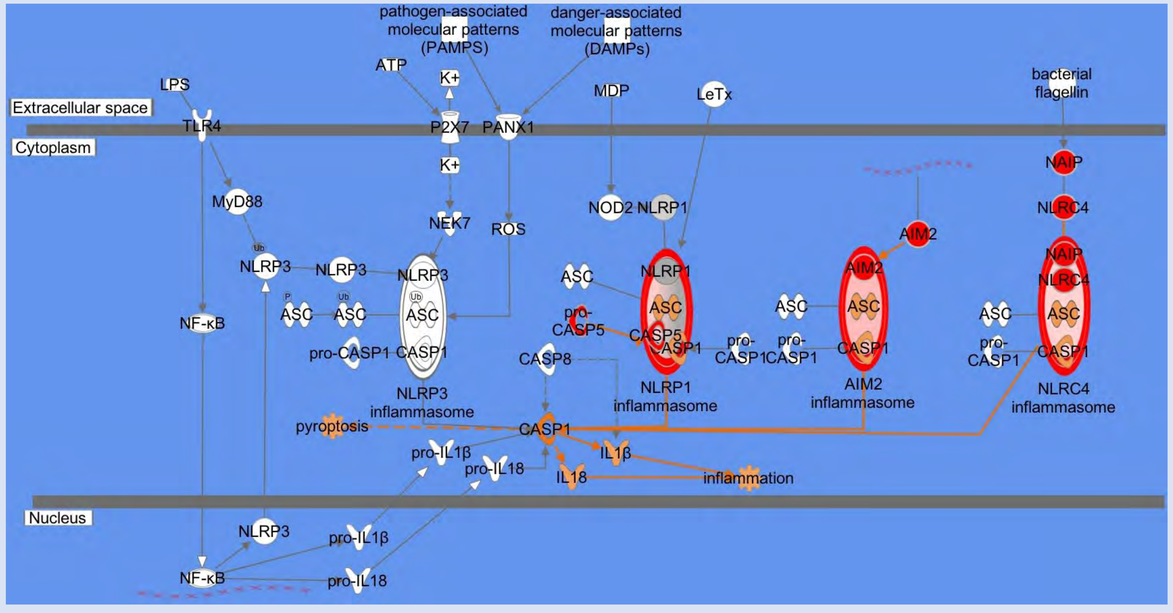


Figure 1. Inflammasome pathway in IRIS

Molecules in red were those found to be differentially abundant in the signatures of TB-IRIS patients when compared to TB-HIV co-infected patients with no IRIS. Molecule activity prediction was performed using IPA to predict downstream effects. Molecules in orange were predicted to be activated, orange arrows show predicted relationships that lead to activation.

Conclusions:

- **Using RNA microarray analysis, transcripts of innate antigen recognition and signaling pathways were over expressed and likely play a crucial role in TB-IRIS.**
- **The use of agents that block inflammasome and IL-1b signaling in recalcitrant TB-IRIS merit further investigation.**

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