M. Tuberculosis-associated Immune Reconstitution Inflammatory Syndrome (IRIS) results in modulation of the innate immune compartment in the absence of generalized T cell activation.

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Abstract

Background:
Immune Reconstitution Inflammatory Syndrome (IRIS) is a paradoxical clinical worsening of a subclinical condition during adequate response to anti-retroviral treatment (ART). IRIS predisposing factors include low pre-ART CD4 counts and robust virological response to ART; however, its pathogenesis remains unclear. We addressed this question studying a group of South African HIV-1-infected subjects presenting with M. Tuberculosis (MTB)-associated IRIS.
Methods:
IRIS definition: ≤ 3 months on ART; documented viral (VL) and CD4 response to ART, acute symptoms incompatible with normal TB presentation or ART-related AE, confirmation of MTB infection.
Subjects: IRIS group: 17 (9 F, 8 M) HIV+ subjects with IRIS presentation (12 pulmonary, 5 extra-pulmonary). Control group: 23 (18F, 5M) HIV+ subjects with similar ART duration. ART regimen: 3TC+D4T+EFV except: 2 IRIS and 1 control, 3TC+D4T+NVP; 1 IRIS, 3TC+ATV+TDF.
Flow cytometry stainings were performed on whole blood samples using custom-designed lyoplates and analyzed on a 4-color flow cytometer (BD Biosciences)
Between-group comparisons were assessed using the Mann-Whitney test; p< 0.05 were considered significant.
Results:
Per study design, pre-ART CD4 count, viral load (VL) and time on ART were similar in the two groups.
At the time of IRIS, CD4 and CD8 counts, but not %, were lower in the IRIS group. IRIS subjects had lower frequency of CD8+ CD28+ (but not CD4+) and CD8+/CD25+/CD62L+ T cells, and higher frequency of CD8+/CD28- T cells. The frequency of CD4 and CD8 T cell activation (CD38+), memory subsets distribution and classic CD4+/CD25+/CD62L+ Treg cells was similar in both groups.
Compared to the control group, IRIS subjects had significantly higher neutrophil count and lower lymphocyte count, hemoglobin and serum Na+ and Cl-. Levels of immature CD161+/CD16-/CD56- NK cells and CD197+ mature myeloid dendritic cells (DC) and NK-like CD56+ T cells were higher in the IRIS group, whereas mature (CD16+) and activated (CD69+ and/or HLA-DR+) NK cells were lower than controls. Other NK and DC subsets were similar in the two study groups.
Conclusions:
We report that MTB-associated IRIS is characterized, at presentation, by expansion and/or redistribution of innate immunity effectors, including myeloid cells (neutrophils and myeloid DC), immature NK cells and NK-like T cells, accompanied by lower lymphocyte counts. This expansion of the innate compartment occurs without evidence of generalized T cell activation.

Methods

Cohort description: >18 y.o. HIV-1 infected subjects attending the CHRU clinic at Helen Joseph Hospital, Johannesburg, RSA, initiating ARV treatment
Treatment: 3TC + D4T + EFV, except: 1 control and 2 IRIS subjects: 3TC + D4T + NVP; 1 IRIS subject: 3TC + ATV + TDF
Study groups:
Group 1: no IRIS, no active TB (n=23); Group 2: TB-related IRIS (n=17)
Diagnosis of TB-related IRIS (at presentation)*:

- Clinical evidence of inflammatory process (e.g. pulmonary involvement, lymphoadenitis etc.)
- Confirmed diagnosis of HIV infection
- Subject is receiving ART for ≤ 3 months
- Clinical presentation not consistent with usual course of new or established TB

Confirmation of initial diagnosis:

- Confirmed diagnosis of active MTB infection (sputum, X-ray and other tests as needed)
- Documented raise CD4 count OR decrease in serum HIV-1 RNA
- Exclusion of ART-related AE as causal agent
- Amelioration or resolution after initiation of anti-TB therapy

* *modified from Shelburne et al [1]*

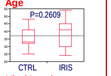
Baseline clinical and demographic characteristics

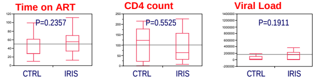
Gender

Group	Females	Males	Total
Control	18	5	23
IRIS	9	8	17
Total	27	13	40

Ethnicity

Group	Black	White	Other
Control	20	0	3
IRIS	14	1	2
Total	34	1	5





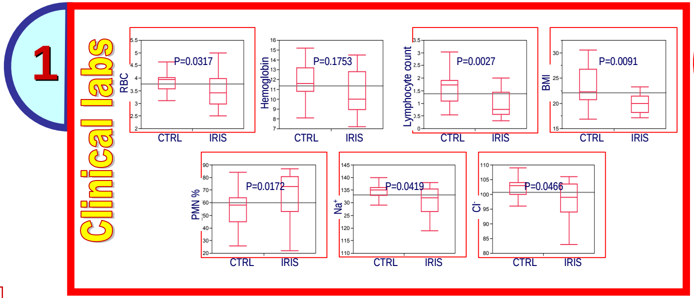
Flow Cytometry
Antibody/fluorochrome combinations (Lyoplates from BD Biosciences)

	FITC	PE	Per-CP-Cy5.5	APC	Target population
1	CD56	CD161	CD3	CD16	NK cell subsets
2	CD56	CD69	CD3	HLA-DR	NK activation
3	CD86	CD303/304 - BDCA2/4	HLA-DR	CD197 - CCR7	DC
4	CD1c - BDCA1	CD11c	CD19	CD197 - CCR7	DC
5	CD38	CD28	CD3	CD4	Activation
6	CD25	CD62L	CD3	CD4	Regulatory
7	CD45RA	CD62L	CD3	CD4	Memory phenotype
8	Ig	Ig	Ig	Ig	Isotype control

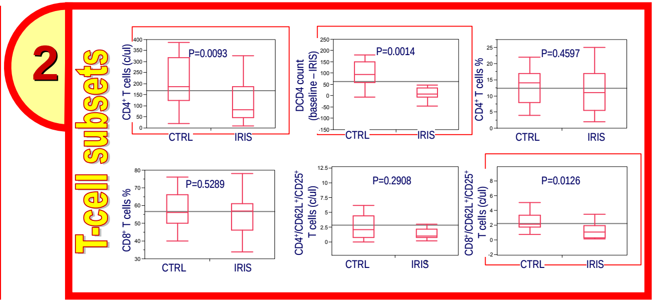
Analysis: FacsCaliber Flow Cytometer using CellQuest software (BD Biosciences).
Statistical methods
Between-groups comparisons (IRIS vs. Control): Mann Whitney test, unadjusted for multiple testing.

Introduction: Immune Reconstitution Inflammatory Syndrome (IRIS) is a paradoxical clinical worsening of a subclinical condition during adequate response to anti-retroviral treatment (ART) [2]. Clinical presentations and pathogens vary: in sub-Saharan Africa, due to high endemic prevalence, M. Tuberculosis infection is the pathogen most commonly correlated with IRIS [3-6].
IRIS predisposing factors include low pre-ART CD4 counts and robust virological response to ART [7]; however, its immunopathogenesis remains unclear. We addressed this question by analyzing the immunophenotype of a group of 17 HIV-1-infected South African with active M. Tuberculosis (MTB)-associated IRIS, as compared to 23 HIV-infected subjects with similar characteristics and undergoing ART for similar extent of time.

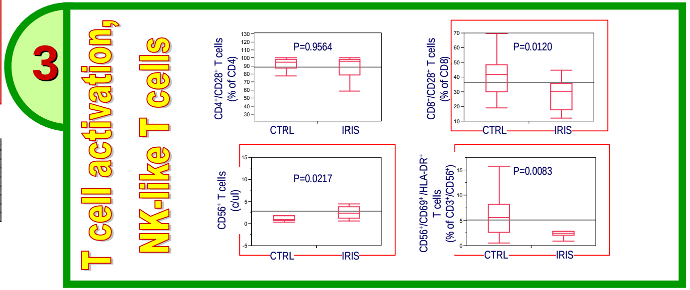
IRIS subjects present with relative anemia and lymphopenia, hyponatremia and neutrophilia as compared to non-IRIS subjects with similar ART duration.



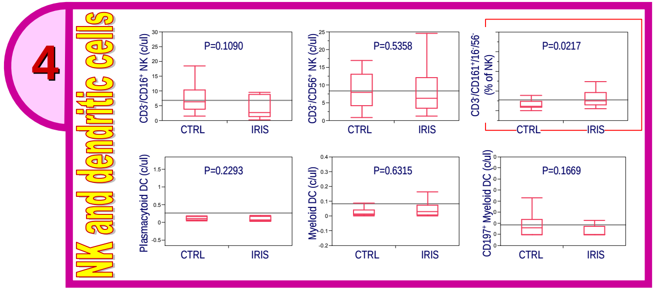
Despite lower CD4 count and post-ART CD4 rise, both CD4 and CD8 frequency are similar in IRIS and non-IRIS subjects. Expression of CD25 andCD62L (T_{reg}) on CD8$^+$ (and possibly CD4$^+$) T cells is decreased IRIS subjects.



Expression of the activation/differentiation marker CD28 is lower in CD8$^+$ (but not CD4$^+$) T cells from IRIS subjects. The frequency of NK-like CD56$^+$T cells is higher in IRIS, but activation (CD69$^+$/HLA-DR$^+$) of these cells is lower.



IRIS is characterized by increased frequency of immature (CD161$^+$/56$^-$) NK cells without loss of mature (CD56+ or CD16+) NK cells.



Conclusions

We have assessed immunologic parameters in a cohort of 17 subjects developing TB-related IRIS after initiation of anti-retroviral treatment and 23 subjects receiving` ART for a comparable time with no IRIS symptom and no TB diagnosis. Per study design, baseline age, time on ART, CD4 and VL were similar in the two groups.
Our data indicate that:

- In addition to signs of inflammation (e.g. neutrophilia, anemia, weight loss), acute IRIS is characterized by lower lymphocyte count and and hyponatremia.
- The lower CD4 counts detected in acute IRIS are attributable to lymphopenia rather that to selective CD4 depletion
- The frequency of CD4$^+$ T_{reg} (CD62L$^+$/CD25$^+$ [8]) was similar in the two groups, but IRIS subject had lower frequency of CD8$^+$/CD62L$^+$/CD25$^+$ T cells, which may have regulatory function [9-10].
- IRIS was not characterized by higher activation of the T cell compartment, but rather by lower expression of CD28 on CD8$^+$ T cells and of CD69 and HLA-DR on CD56$^+$ NK-like T cells
- Immature (CD161$^+$/56$^-$/16$^-$) NK cells [11-12] were higher in IRIS subjects. Other NK subsets, as well as dendritic cell subsets, were similar in the two study groups.

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