Abstract #:222

Inflammasome and Pyroptosis are Involved in The Lack of Immune Response During cART

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Abstract

Background: Inflammasome-mediated activation of caspase-1 regulates inflammatory responses and pyroptosis. Pyroptosis was recently shown to play a major role in CD4 T lymphocyte loss and to contribute to immune activation in HIV infection.The possible role of inflammasomes and pyroptosis in the lack of immune reconstitution seen in a percentage of ART-treated HIV patients has nevertheless not been investigated. We analyzed possible associations between inflammasome activity, caspase-1 activation, pyroptosis and immune reconstitution in HIV+ ART treated patients

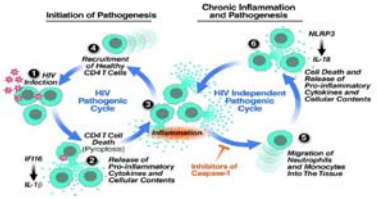
Methods: Cross-sectional, single-site study. HIV-infected patients on antiretroviral therapy for ≥24 months and plasma HIV-RNA<50 cp/mL for ≥12 months, matched for nadir CD4 T cell count were enrolled. Presence of opportunistic AIDS-related diseases, HBV or HCV coinfection, chronic inflammatory disorders, ongoing immunosuppressive therapy were exclusion criteria. Patients were classified as immunological responders (IR) or non responders (INR) if CD4 count was ≥500 or ≤350 cells/μL, respectively. Expression of inflammasome, caspases 1, 3, 4, and 5, pro-inflammatory cytokines and of IFI16 genes was measured in unstimulated and in AT2-HIV-1 stimulated cells of all IRs and INRs.

Results: 39 patients (22 IRs; 17 INRs, 77% M, medians: age 47 years, time from HIV diagnosis 10 years, time with HIV-RNA<50cp/mL 57 months) were enrolled. INR patients were older (median 60 *vs* 43 years, p<0.001) and had a higher prevalence of past AIDS-defining illnesses (76% *vs* 18%, p<0.001). Median CD4 count was 840 (IQR 718-1131) cells/μL in IRs *vs* 295 (IQR 256-343) cells/μL in INRs. AT2 stimulation induced NLRP3 gene expression in both IRs and INRs; NLRP3 and IL-18 expression were nevertheless significantly increased in INRs compared to IRs (p=0.009 and p=0.004). Significant higher caspase-1 expression was seen as well in both unstimulated (p=0.02) and AT2-stimulated cells of INRs (p=0.003), whereas caspase 3, 4 and 5 expression was similar in both groups. Finally, IFI16 expression as well plasma concentration of caspase-1 and IL-1β were higher in INR compared to IR patients.

Conclusions: Increased inflammasome and caspase-1 activation is observed in INR patients. The upregulation of these proinflammatory mechanisms plausibly contributes to the persistent immune activation that characterize INRs. Notably, caspase-1 activation is likely to induce CD4 T cell loss via pyroptosis, contributing to the unsatisfactory CD4 recovery seen in INRs.

Background

- 6 to 20% of ART-treated patients commonly referred to as 'immunological non-responders' (INR), fail to achieve a significant immune recovery despite virological response to treatment.
- INRs display higher levels of inflammation and immune activation compared to immunological responder (IR) patients and have higher risk of of AIDS progression, non-AIDS-related morbidity and death (*Weber et al. 2006; Choi et al. 2007; Kirk et al. 2007; Triant et al. 2007; Engels et al. 2008; Joshi et al. 2011*).
- Inflammasomes, such as NLRP3, recognize pathogens and trigger immune responses by activating caspase-1, which, in turn, leads to the production of pro-inflammatory cytokines IL1β and IL-18.
- Caspase-1 activation leads also to pyroptosis, a form of programmed cell death charactherized by high levels of inflammation.
- Pyroptosis was recently shown to play a major role in CD4 T lymphocyte loss and to contribute to immune activation in HIV infection even in absence of ongoing HIV-replication. (*Doitsh G et al., 2014; Doitsh G et al., 2016*)
- The role played by caspase-1 and pyroptosis in immunological failure has not yet been investigated.



Doitsh G. et al., 2016

Materials and Methods

Study Population and Inclusion Criteria:

39 HIV-infected ART-treated patients matched for CD4+ nadir, were enrolled at the Unit of Infectious Diseases, San Gerardo Hospital, Monza, Italy. 22 were Immunological responders (IR) with lymphocyte T CD4+ count >500 cells/μL and 17 were immunological non responders (INR) patients with lymphocyte T CD4+ count <350. The inclusion criteria were: men and women >18 of age, HIV positivity tested with ELISA and confirmed by Western Blot, combined antiretroviral therapy (ART), duration of ART > 24 months, plasma HIV-RNA < 50 cp/mL for at least 12 months. Exclusion criteria were co-infection with HCV or HBV, ongoing immunosuppressive therapy and chronic inflammatory disorders

Analyses performed:

- Inflammasome pathway gene expression in AT2-treated-HIV_{1Bal}-stimulated PBMCs using a Real-Time PCR array including a set of 84 optimized primers on 96-wells plate (Real-Time PCR)
- Real-Time PCR to evaluate the gene expression of caspase-1, caspase-3, caspase-4, caspase-5, IFI16 and pro-inflammatory cytokines
- ELISA assays to evaluate caspase-1 and IL-1β levels in plasma from INRs and IRs.
- ELISA assays to evaluate microbial translocation markers (sCD14 and LPS) in plasma from INRs and IRs.

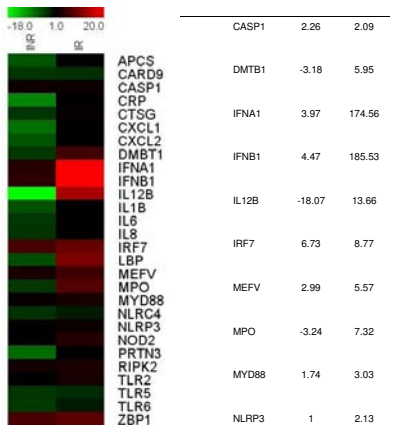
AIM

To investigate possible associations between caspase-1 activation, pyroptosis and the degree of immune reconstitution in chronic infected HIV+ subjects receiving ART

Results

	IR (n=22)	INR (n=17)	P
Age	43 (40-48)	60 (50-66)	<0.001
Male gender	16 (72.7)	14 (82.4)	0.704
Caucasian	19 (86.4)	14 (82.4)	1.000
Riskfactor:			0.769
Heterosexual	11 (50)	10 (58.8)	
Homosexual	5 (22.7)	4 (23.5)	
Unknown	6 (27.3)	3 (17.6)	
Years from HIV diagnosis	9.3 (6.2-20.2)	10.2 (3.8-15.6)	0.557
Past AIDS-defining events	4 (18.2)	13 (76.5)	<0.001
Current CD4	840 (718-1131)	295 (256-343)	<0.001
Current CD4%	34 (28-40)	19 (16-21)	<0.001
Current CD8	1139 (781-1383)	685 (489-899)	0.001
Current CD8%	41 (36-47)	48 (37-53)	0.300
Current CD4/CD8	0.81 (0.60-1.03)	0.41 (0.29-0.58)	<0.001
Current CD4/CD8 >1	6 (27.3)	0	0.027
Months from last VL>50	53.7 (33.4-67.7)	59 (25.9-84.9)	0.617
Years from ARTinitiation	7.7 (4.8-11.2)	10.2 (3.3-13.4)	0.540
Months from last regimen initiation	48.5 (18.2-60.9)	16.8 (5.5-41.2)	0.209
NRTI	20 (90.9)	16 (94.1)	1.000
NNRTI	12 (54.5)	7 (41.2)	0.613
PI	6 (27.3)	6 (35.3)	0.851
InSTI	6 (27.3)	7 (41.2)	0.568
EI	0	0	nc

Table 1: Demographic and clinical characteristics of patients enrolled in the study. Median, interquartile ranges, and *p* values are shown.



Nfold >2	Gene	INR	IR
	CASP1	2.26	2.09
	DMTB1	-3.18	5.95
	IFNA1	3.97	174.56
	IFNB1	4.47	185.53
	IL12B	-18.07	13.66
	IRF7	6.73	8.77
	MEFV	2.99	5.57
	MPO	-3.24	7.32
	MYD88	1.74	3.03
	NLRP3	1	2.13

Figure 1: Inflammasome pathway gene expression in AT2-$HIV1_{Bal}$-stimulated PBMCs. mRNA expression of 84 genes of the inflammasome pathway has been assessed by real-time quantitative RT-PCR, calculated relative to two housekeeping genes and shown as fold-change expression from the untreated sample. Gene expression (nfold) is shown as a color scale from green to red (-18 to +20) (MEV multiple experiment viewer software). Only targets showing > 2-fold modulation are considered significant and are shown in table.

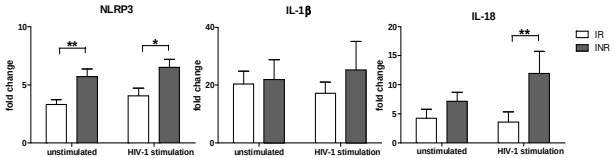


Figure 2: Gene expression of proinflammatory cytokines (IL-1β and IL-18) and inflammasome NLRP3.
Gene expression in AT2-$HIV1_{Bal}$-stimulated PBMCs of IR and INR individuals. Fold change mean values and SEM are shown. **p*<0.05, ***p*<0.01.

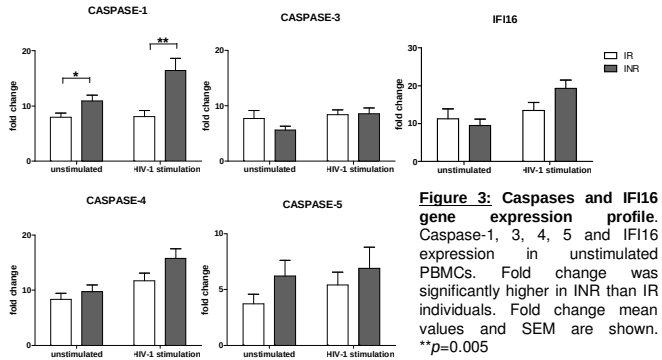


Figure 3: Caspases and IFI16 gene expression profile. Caspase-1, 3, 4, 5 and IFI16 expression in unstimulated PBMCs. Fold change was significantly higher in INR than IR individuals. Fold change mean values and SEM are shown. ***p*=0.005

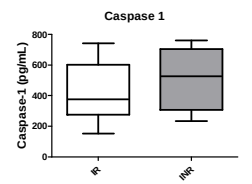


Figure 4: Plasma quantification of caspase-1 in IR and INR patients. Caspase-1 expression in IRs (left) and INRs (right). Median values and max and min values of distributions are shown.

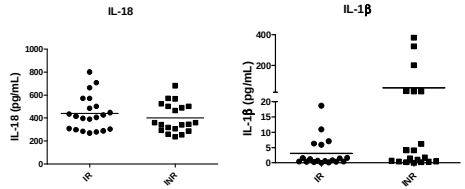


Figure 5: IL-18 and IL-1β plasma quantification in IR and INR patients. IL-18 and IL-1β expression in IRs (dots) and INRs (squares). Horizontal lines indicate mean values.

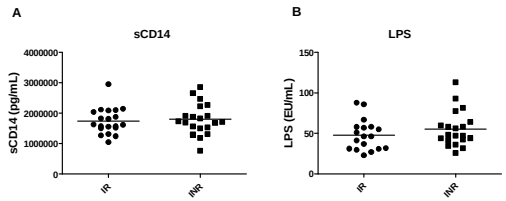


Figure 6: sCD14 and LPS quantification in plasma from INRs and IRs. sCD14 (Panel A) and LPS (Panel B) plasma expression in IRs (dots) and INRs (squares). Horizontal lines indicate mean values

Conclusions

✓ Increased inflammasome and caspase-1 activation is observed in INR patients.
✓ The upregulation of these proinflammatory mechanisms plausibly contributes to the persistent immune activation that characterize INRs.

Caspase-1 over-expression suggests that it may be able to induce CD4 T cell loss via pyroptosis, contributing to the unsatisfactory CD4 recovery observed in INRs.