Clinical Immunology 209 (2019) 108266

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Antagonistic role of IL-1ß and NLRP3/IL-18 genetics in chronic HIV-1 infection

Edione C. Reis[a], Vinicius N.C. Leal[a], Lais T. da Silva[b], Marilia M.L. dos Reis[c], Enrique R. Argañaraz[c], Telma M. Oshiro[b], Alessandra Pontillo[a,*]

[a] Laboratório de Imunogenética, Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo/USP, São Paulo, SP, Brazil
[b] Laboratório de Investigação em Dermatologia e Imunodeficiências - LIM 56, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de São Paulo, SP, Brazil
[c] Laboratório de Virologia Molecular, Faculdade de Ciências da Saúde, Universidade de Brasília/UNB, Brasília, DF, Brazil

ARTICLE INFO

Keywords:
HIV-1, chronic infection
NLRP3
Inflammasome
IL-1ß
IL-18

ABSTRACT

Host genetics affects both susceptibility and progression of HIV-1 infection. NLRP3 inflammasome provides a first-line defense in viral infections, and, accordingly, gain-of-function variants in *NLRP3* have been associated with protection against HIV-1.

Despite antiretroviral treatment (ART), HIV-infected patients continue to present systemic inflammation with a heterogeneous prognosis. As NLRP3 inflammasome is involved in several chronic diseases by amplifying "sterile" inflammation, its role in chronic phase of HIV infection has been postulated.

Little is known about inflammasome genetics in HIV-infected patients and whether it may play a role in the different clinical outcomes. Therefore, we questioned whether NLRP3 inflammasome genetics could affect the clinical course of HIV-1 infection as it does in host/virus interaction.

For this purpose, we analyzed selected single nucleotide polymorphisms (SNPs) in ART-treated HIV-infected patients ($n = 300$), in Long Term Non-Progressors/Elite Controllers and progressors ($n = 133$), and in HIV-infected individuals submitted to dendritic cell (DC)-based immunotherapy ($n = 19$).

SNPs leading to increased activation of NLRP3 inflammasome are beneficial for patients, while SNPs that negatively affect NLRP3 activation or IL-18 production, detrimental. In contrast, gain-of-function variant in *IL1B* is also detrimental for patients, suggesting that while IL-1ß possible contributes to immune exhaustion, the axis NLRP3-inflammasome/IL-18 could act positively in chronic infection. Functional assays supported genetic results: *NLRP3* variants associated with good quality HIV+ DC, and *IL1B* -511C > T with a poor one. Loss-of-function SNPs affect HIV+ T cells proliferation.

These findings proposed for the first time that NLRP3 inflammasome, mainly through IL-18, play a protective role in chronic HIV infection.

1. Introduction

To counteract infections, several cytoplasmic innate immune receptors (i.e.: NLRP3, NLRC4, AIM2) are known to induce the assembling of the multiprotein complex termed inflammasome, which lead to the activation of inflammatory caspase-1 and the consequent cleavage of several substrates including the pro-inflammatory cytokines IL-1ß and IL-18, and the cell death pyroptosis protein, gasdermin D [1]. On the other side, inflammasome could be improperly activated in sterile condition causing severe chronic inflammatory disorders, such as autoimmunity, obesity and cardiovascular diseases [2].

Some inflammasome receptors (i.e.: NLRC4 and AIM2) directly recognize molecular patterns both from pathogens (PAMPs) or from host tissue damage/stress (DAMPs), while others, such as NLRP3, can be indirectly activated by several PAMPs and DAMPs through the induction of cytoplasmic alterations, such as ATP-dependent K+ efflux (by P2X7 receptor), lysosomal or mitochondrial damage [1].

Gain-of-function polymorphisms in NLRP3 (i.e.: missense substitution p.Q705K/rs35829419; 3'UTR variant rs10754558) lead to the decrease of inflammasome activation threshold and a general increase of IL-1ß and IL-18 production [3,4]. Loss-of-function variants in NLRP3 regulators could increase inflammasome activation, such in the case of the non-sense variation rs2043211 (Cys10Ter) in NLRP3 inhibitor CARD8 [5], or decrease it, as for missense substitutions in P2X7 (as

* Corresponding author at: Avenida Prof. Lineu Prestes, 1730, Cidade Universitária, São Paulo, SP, Brazil.
E-mail address: alepontillo@usp.br (A. Pontillo).

https://doi.org/10.1016/j.clim.2019.108266
Received 28 August 2019; Received in revised form 23 September 2019; Accepted 2 October 2019
Available online 24 October 2019

Table 1
Demographic and clinical characteristics of HIVinfected cohorts. Sex, age, time of diagnosis (total and before the treatment) as well as plasma viral load (PVL) and CD4+ T cells counts before (0) and after [1] the treatment (ART or DC-based immunotherapy) were reported. For CD4+ T cells mean level and nadir of values collected during follow-up were indicated. Data are expressed as mean ± standard deviation (SD), except where alternatively indicated. LTNP: long-term non progressors; EC: elite controllers; P: progressors. TB: tuberculosis.

Cohort ID	Cohort 1	Cohort 2		Cohort 3
Subjects, n	300	133		19
Sub-groups, (n)	–	LTNP/EC (84)	P (49)	–
Sex (M/F), n	187/113	58/26	36/13	14/5
Age (years), mean ± SD	52.22 ± 10.95	43.06 ± 11.55	37.33 ± 10.78	34.37 ± 7.97
Time of diagnosis (years), mean ± SD	19.43 ± 5.74	10.92 ± 7.02	5.47 ± 3.57	8.37 ± 12.16
Time of diagnosis before treatment (years), mean ± SD	0.85 ± 1.94	–	–	–
Subjects with non-HIV morbidity, n	52	0	0	0
PVL_0 (log RNA copies/mm^3)	4.55 ± 1.10	2.50 ± 1.65	3.54 ± 1.66	4.16 ± 0.62
PVL_1 (log RNA copies/mm^3)	≤ 1.7	–	–	3.80 ± 1.01
CD4+ T_0 (cells/mm^3), mean ± SD	322.5 ± 278.3	609.4 ± 213.3	314.6 ± 244	452.0 ± 102.1
CD4+ T_1 (cells/mm^3), mean ± SD	755.4 ± 471.0	–	–	471.7 ± 135.8
CD4+ T_1 (cells/mm^3), mean nadir ± SD	403.9 ± 229.5	–	–	–

largely reviewed by Di Virgilio et al. [6,7]). As expected, due to NLRP3 inflammasome role in innate immunity and inflammation, these polymorphisms have been associated with protection against microbes [8–11] or eventually to susceptibility to chronic sterile diseases [8,12].

Previous results from our group have shown the protective effect of *NLRP3* rs10754558 3'UTR gain-of-function variant on HIV-1 susceptibility [13,14], suggesting an important role of NLRP3 during the first contact with HIV. Subsequent studies demonstrated that the virus is able to activate NLRP3 inflammasome in monocytes [15] and dendritic cells (DC) [16] resulting in IL-1ß and IL-18 production, innate immune response and inflammation at the site of infection.

Altogether, these findings indicate a role of NLRP3 inflammasome in the first line defense against HIV-1. However, poor data are nowadays available about the role of the complex in the chronic infection and the resulting immune system exhaustion of HIV-infected patients. Despite antiretroviral treatment (ART), HIVinfected patients continue to present systemic inflammation with heterogeneous prognosis (i.e.: cardiovascular diseases, metabolic disorders and neurologic impairment) [17]. Since NLRP3 inflammasome is dysregulated in several chronic diseases, such as metabolic disorders and obesity, neurodegenerative diseases, autoinflammatory syndromes and autoimmune diseases [2], in which it plays a pivotal function amplifying "sterile" inflammation, a role in chronic phase of HIV infection also have been postulated [18,19]. Several molecules were developed to specifically inhibit different checkpoints of inflammasome activation [20,21]. Pharmacologic inhibition of inflammasome could represent a targeted treatment for chronic complications in HIV-infected patients, and the demonstration of inflammasome contribution is an appealing challenge.

As above-mentioned, genetic variants in inflammasome components and/or regulators modulate the threshold of complex activation and contribute to the heterogeneity of clinical manifestations of specific infectious or sterile diseases, leading to the use of inflammasome inhibitors in genetic positive individuals [21].

Although it was demonstrated an important role of inflammasome genetics during the first contact with HIV [13,14], little is known about inflammasome genetics in HIV-infected patients and whether it may play a role in the different clinical outcomes. Therefore, we questioned whether NLRP3 inflammasome genetics could affect the clinical course of HIV-1 infection as it does in host/virus interaction.

Whether at the first host/virus interaction the activation of NLRP3 inflammasome seems to be important to counteract HIV-1 acquisition [15,16], we hypothesize that, once the infection is established, the activation of inflammasome, leading to the production of pro-inflammatory cytokines IL-1ß and IL-18, and/or eventually contributing to immune cells loss through pyroptosis, may be disadvantageous rather than beneficial.

Considering that host genetics affect both the susceptibility as well as the progression of HIV infection, we then questioned whether inflammasome genetics could affect the clinical course of HIV-1 infection.

For this purpose, we evaluated the effect of functional NLRP3 inflammasome genetic variants on plasma viral load and T lymphocytes level in a cohort of HIV+ chronically infected patients. We then replicated the study in a cohort of patients classified as Long Term Non-Progressors (LTNP) or Elite Controllers (EC) and progressors (P), and in a group of HIV-infected individuals submitted to DC-based immunotherapy. These last two cohorts help us to refine our results by elucidating the contribution of inflammasome genetics in individuals that naturally control HIV infection (LTNP/EC), or better respond to immunization, as an indirect measure of immune system preservation/exhaustion of HIVinfected patients.

2. Patients and methods

2.1. Cohort 1: São Paulo (SP) HIV cohort

300 HIV-infected patients (52.22 ± 10.95 years), proceeding from the metropolitan area of São Paulo (SP, Brazil), seropositive for at least 5 years (19.43 ± 5.74 years), in antiretroviral therapy were recruited for genomic DNA isolation at the service for HIV-infected patients health care (SEAPH) of "Hospital das Clinicas" and Faculty of Medicine, University of São Paulo (HC-FMUSP; SP, Brazil) from January 2016 to December 2018. Plasma HIV RNA levels and CD4+ T cell counts were measured either before the start of ART and during the course of clinical care by the clinical laboratory associated with the medical center. As all the patients were nowadays in ART, we estimated the genetic association taking into account the clinical data before and after the start of ART (mean or nadir values obtained from follow-up data). 52 out of 300 patients present of at least one of non-HIV diseases (i.e.: tuberculosis, opportunistic infection, osteoporosis, cardiovascular diseases, autoimmune diseases, renal or liver failure). Demographic characteristics of the participants, as well as PVL and T lymphocytes counts before and after the start of ART are summarized in Table 1.

2.2. Cohort 2: long-term non-progressors (LTNP), elite controllers (EC) and progressors (P)

Genomic DNA of 133 HIV infected patients (43.06 ± 11.55 years) naïve for ART and grouped according to clinical disease progression in EC (50–1000 copies HIV genome/mm^3, and > 400 CD4+ T lymphocytes/mm^3), LTNP (< 10,000 copies HIV genome/mm^3, and > 400 CD4+ T lymphocytes/mm^3), and P (> 10,000 copies HIV genome/mm^3, and < 200 CD4+ T lymphocytes/mm^3) was included in the study. Main characteristics of this cohort are described in [22] and summarized in Table 1. For genotyping analysis we grouped EC and

LTNP ($n = 84$) and P ($n = 49$).

2.3. Cohort 3: Immunized HIV-infected patients

Genomic DNA and peripheral blood samples of 19 HIV-infected patients from metropolitan area of São Paulo (SP, Brazil) and submitted to DC-immunotherapy at the Laboratory of Medical Investigation (LIM)-56 of HC-FMUSP (phase I/II clinical trial; clinical trial.gov ID: NCT02766049) were used for genotyping and cellular assays. All patients were adult (34.37 ± 7.97 years), seropositive for at least 5 years (8.37 ± 2.16), naïve for ART for at least 1 year, and without clinical AIDS or other infectious or chronic diseases, with blood $CD4^+$ T cells count > 350 cells/mm^3, and PVL > 1000 copies HIV genome/mm^3 before the start of the immunization. They were vaccinated with three doses of 3–30 × 10^6 autologous HIV-pulsed DC in 2-week intervals (according to phase-I trial [23]) and followed-up for 1 year. Complete data of the trial are not yet published, however the immunization product resulted safe, with no adverse events, but with a modest effect on PVL and T cell count. Main characteristics of the participants, as well as PVL and T lymphocytes counts before and after 1 year of the immunization, are summarized in Table 1.

2.3.1. Ethical statement

All subjects gave informed consent and the research protocols were approved by the corresponding institutional review boards on the conduct of research human subjects at each study site.

2.3.2. Laboratory analysis

The plasma viral load was quantified using the VersantR HIV-1 RNA 3.0 Assay bDNA kit (*Bayer Corporation*) and System 340 bDNA. The T lymphocytes count was performed using CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC with BD Trucount™ Tubes by flow citometry (FACSCalibur) *(BD Biosciences)*.

2.3.3. SNPs genotyping

Eight single nucleotide polymorphisms in NLRP3 inflammasome components and related cytokines were selected based on literature and on previous results, as detailed in Table 2. Genotyping was performed by commercially available allele-specific TaqMan assays (*Applied Biosystems, Thermo Fisher Scientific*) and qPCR on a QuantStudio 3.0 real-time PCR instrument (*Applied Biosystems*). Allelic discrimination was performed using the QuantStudio 3.0 software (*Applied Biosystems*).

2.3.4. Cellular functional assays

Peripheral blood of HIV-infected patients belonging to cohort 3 was collected for monocytes-derived DC obtainment before the start of immunization protocol. Briefly, peripheral blood mononuclear cells (PBMC) were isolated from fresh blood by Ficoll-Paque gradient (*GE Healthcare*), following the manufacturer's protocol. PBMC were used for monocytes and lymphocytes obtainment. Monocytes were cultured at 1.5–2 × 10^6 cells/ml in AIM-V medium (*Gibco, Thermo Fisher Scientific*) containing 50 ng/ml GM-CSF (*Cell-Genix*) and 50 ng/ml IL-4 (*Cell-Genix*) for 5 days to differentiate in immature DC (iDC). Lymphocytes were frozen and stored to be used for autologous DC-lymphocytes co-culture assays. DC were pulsed with autologous aldrithiol-inactivated HIV-1 (10^6 viral particles/30 × 10^3 DC) for 4 h, and then treated with 50 ng/ml IL-4, 50 ng/ml GM-CSF, 50 ng/ml TNF, 10 ng/ml IL-1ß, 100 ng/ml IL-6 for 48 h to become mature DC (mDC). DC maturation and activation were analyzed by the expression of cell surface markers (CD14, CD11c, HLA-DR, CD80, CD86, CD83, CD40, DC-SIGN, CCR6) and flow cytometry (FACSCalibur) *(BD Biosciences)*. Cell viability was determined by the use of LIVE/DEAD® kit (Thermo Fisher Scientific) and flow cytometry (FACSCalibur) *(BD Biosciences)*. Phagocytosis capacity of iDC was evaluated by cell internalization of fluorescently labelled (FITC) dextran beads and flow cytometry.

Ability of mDC to activate T cells was tested by mDC/autologous T lymphocytes co-culture assays. Briefly, 4 × 10^4 mDC were cultured with 2 × 10^5 autologous T lymphocytes (ratio: 1/5) in duplicates in 96-round bottom wells plates for 24 and 96 h. Brefeldin A (20 µg/ml; *Sigma-Aldrich*) was added 6 h before the end of assay. T lymphocytes proliferation was measured by the mean of CSFE dilution (*Thermo Fisher Scientific*). IFN-γ, MIP-1β, IL-2 and TNF production by T lymphocytes were measured by intracellular staining and flow cytometry (FACSCalibur) *(BD Biosciences)*.

2.3.5. Statistical analysis

Multivariate analysis and general linear model (GLM) were used to analyse the SNPs associated with clinical or laboratory variables ("SNPassoc" package; www.r-project.org). Data were adjusted for sex, age, time from diagnosis and presence of non-HIV chronic diseases (yes/no). Akaike information criterion (AIC) was used for the selection of the best fitting model of inheritance of each SNP [24]. Haploview software [25] was used to investigate the linkage disequilibrium (LD) pattern and for deriving the haplotypes. The Fig. 1 showed the LD blocks and the haplotypes for blocks. Rare haplotypes (< 0.05) were excluded from analysis. Taking into account that *IL1B* and *IL18* SNPs share the same genetic locus (2q13-q21), and that 2 SNPs of *NLRP3* and *P2X7* also are supposed to present a LD, a formal Bonferroni correction for the number of independent genetic *loci* would require a significance threshold of $p = .013$ (p_0/n, $p_0 = 0.05$, $n = 4$ independent *loci*). Multi *t*-test was applied in time-course analysis.

3. Results

Multivariate analysis of inflammasome SNPs distribution in HIV-infected patients (cohort 1) was performed according to PVL and CD4+ T lymphocytes count before and after ART (the arithmetic mean and nadir of 10-years follow-up have been used). Sex, age and time of disease before ART or total time of disease, respectively, were included in the analysis as confounder variables. Complete analysis is included in Supplementary File 1. Statistically significant results are resumed in Table 3.

Both *IL18 v*ariants resulted significantly associated with PVL before the start of ART, being more frequent in patients with a high PVL_0, according to a dominant model of inheritance for the minor allele (rs1834481 C/G + G/G: 5.0 ± 0.1, versus C/C: 4.4 ± 0.1; and rs5744256 A/G + G/G: 5.0 ± 0.1, versus A/A: 4.1 ± 0.1) (Table 3).

Accordingly, the haplotype containing minor alleles of both SNPs (rs1834481/G-rs5744256/G; frequency: 0.16) was significantly associated to increased PVL_0 when compared to the wild type one (rs1834481/A-rs5744256/C: frequency: 0.83) (G-G: 4.8 ± 0.3 log RNA copies/ml, versus A-C: 3.8 ± 0.1 log RNA copies/mm^3; $p_{adj} = 0.006$).

These two variants (analyzed alone or in haplotypes) did not associate to CD4+ T cells count before ART ($p_{adj} > 0.05$), nor with clinical variables after ART ($p_{adj} < 0.05$) (Supplementary File 1). *IL18* haplotypes did not associate with PVL_1, or CD4+ T cells count.

Differently from *IL18* SNPs, the gain-of-function promoter variant *IL1B* rs16944 (previously known as −511C > T) resulted differently distributed according to CD4+ T cells level in ART treated patients (CD4+ T_1) being more frequent in patients with a lower lymphocytes' count after the start of the treatment (T/T: 538.4 ± 39.0 cells/mm^3; C/C + C/T: 660.1 ± 20.5; according to a recessive model of inheritance for the minor T allele (Table 3). Moreover, during follow-up, rs16944 T/T carriers showed a reduction of CD4+ T cells level compared to non-carriers (C/C + C/T) ($p = .001$) (Fig. 2).

P2X7 rs2230911 (Ser357Thr) resulted significantly associated with PVL in HIV-infected individuals before the start of ART, being G/G genotype more frequent in patients with higher PVL_0 (G/G: 6.2 ± 0.2 log RNA copies/mm^3), than C/C or C/G (C/C + C/G: 4.5 ± 0.1 log RNA copies/mm^3), according to a recessive model of inheritance for the minor G allele (Table 3). The same polymorphism resulted associated to lower plasma level of CD4+ T_0 (G/G: 27.5 ± 26.5 cells/mm^3) than C/

Table 2
Genetic variants in inflammasome components. Identification number of polymorphism (ID), gene and RefSeq sequences, chromosomic position (Position; from GRCh38.p12 sequencing release), nucleotide and amino acidic change (Variation), functional effect (Effect) and relative reference (Ref), as well as minor allele frequency (MAF) in different populations (EUR: european-ancestral; AFR: african-ancestral; SAS: south asian-ancestral; EAS: east-asian ancestral; AMR: amerindian-ancestral; from 1000Genome project) are reported. For each gene, the chromosomic locus, the mRNA and protein reference sequences are indicated. Data about functional effect of polymorphism were obtained from literature, and/or from public databases of gene/protein expression (https://www.GTEX.org; and/or https://www.proteinatlas.org).

Gene (RefSeq)	ID	Position (GRCh38.p12)	Variation	Effect	EUR	AFR	SAS	EAS	AMR
NLRP3 NG_00709.2 NM_004895.4	rs35829419	247425556	g.247425556C > A c.2113C > A p.(Gln705Lys)	Increased IL-1ß/IL-18 production [3]	0.05	0.01	0.04	0	0.02
	rs10754558	247448734	g.247448734 G > C c.*230 G > C	Higher *NLRP3* mRNA stability; [4] lower miR223, miR4273 binding	0.46	0.25	0.37	0.41	0.25
IL1B NG_008851.1 NM_000576.2	rs16944	112837290	g.112837290 A > G c.-598 T > C	Increased IL-1ß release [23]	0.35	0.57	0.60	0.47	0.55
IL18 NG_028143.1 NM_001562.4	rs5744256	112152125	g.112152125 A > G	Lower plasma level of IL-18 [26]	0	0.22	0.06	0	0.10
	rs1834481	112153104	g.112153104C > G	Lower plasma level of IL-18 [29]	0.20	0	0.06	0	0.10
CARD8 *NG_029574.2* *NM_001184900.3*	rs2043211	48234449	g.48234449 A > T c.304 T > A p.(Phe102Ile)	Increased IL-1ß release Higher *CARD8* expression* [5]	0.33	0.18	0.32	0.51	0.27
P2X7R *NG_011471.2* *NM_002562.6*	rs3751143	121184501	g.121184501A > C	Loss of function – suboptimal receptor assembly affecting pore formation [7]	0.20	0.08	0.32	0.27	0.19
	rs2230911	121177328	g.121177328C > G	Loss of function – partial reduction in channel and pore formation [7]	0.08	0.16	0.19	0.17	0.26

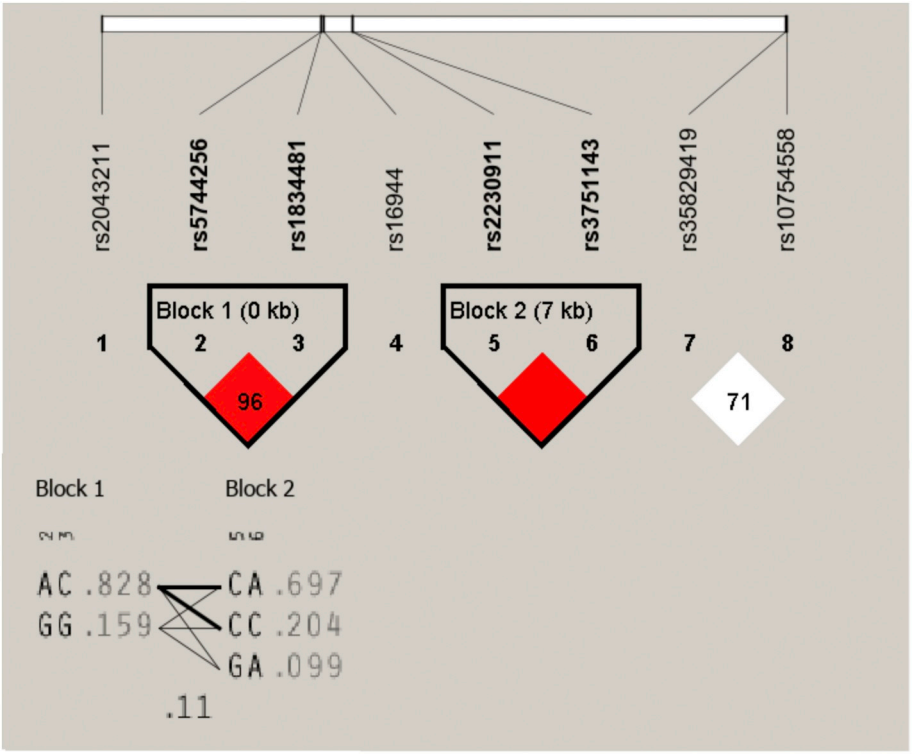

Fig. 1. Linkage Disequilibrium analysis results for inflammasome SNPs in the studied cohort. Haploview plot shows haploblocks and Linkage Disequilibrium (LD) values (reported as D'/LOD) for inflammasome SNPs genotyped in cohort 1.

C or C/G (C/C + C/G: 331.2 ± 19.2 cells/mm^3) according to a recessive model of inheritance (Table 3). Differently, rs3751143 (Glu496Ala) was not associated with PVL_0 and CD4 + $_0$ T cells count (p_{adj} > 0.05) (Supplementary File 1). None of those SNPs significantly associated with clinical data after ART (Supplementary File 1). Accordingly, *P2X7* haplotypes did not resulted significantly associated to PVL nor to CD4+ T cells count (data not shown).

NLRP3 variants rs35829419 (Gln705Lys) and rs10754558 (3'UTR)

Table 3
Multivariate analysis results for inflammasome SNPs association with HIV-infected patients clinical variables. Multivariate analysis was performed for inflammasome SNPs and plasma viral load (PVL) and CD4 T cells count (CD4+ T) before and after antiretroviral therapy (ART). Sex, age, time of disease and presence of non-HIV morbidities were included in the analysis as confounder variables. Only statistically significant results after correction for multiple comparisons ($p < .013$) are reported. Gene, single nucleotide polymorphism (SNP) or SNPs haplotype, genotypes and haplotypes distribution (frequency), mean ± standard error (SE), adjustes *p*-values(p_{adj}) are reported for plasma viral load before (PVL_0) and after (PVL_1) the start of ART, and for CD4+ T cells count before (CD4+ T_0) and after (CD4+ T_1) the start of ART.

Clinical variable	Gene	SNP/haplotype (minor allele)	Genotypes /Haplotypes (frequency)	Mean ± SE	p_{adj}
			Before ART		
PVL_0 (log RNA copies/mm^3)	*IL18*	rs1834481 (G)	C/C (0.68) C/G (0.30) G/G (0.02)	4.4 ± 0.1 5.0 ± 0.1 4.9 ± 0.3	0.005
		rs5744256 (G)	A/A (0.70) A/G (0.27) G/G (0.03)	4.1 ± 0.1 4.9 ± 0.1 5.2 ± 0.3	0.006
		rs1834481(G)-rs5744256 (G)	others (0.83) /G−/G (0.16)	4.3 ± 0.1 4.8 ± 0.3	0.006
	P2X7	rs2230911 (G)	C/C (0.81) C/G (0.18) G/G (0.01)	4.6 ± 0.1 4.1 ± 0.3 6.2 ± 0.2	0.012
CD4+ T_0 (cells/mm^3)	*P2X7*	rs2230911 (G)	C/C (0.84) C/G (0.16) G/G (0.01)	288.1 ± 18.4 424.6 ± 49.5 27.5 ± 26.5	0.008
			After ART		
CD4 + T_1 (cells/mm^3)	*IL1B*	rs16944 (T)	C/C (0.34) C/T (0.51) T/T (0.15)	724.8 ± 41.3 652.5 ± 24.7 497.8 ± 38.2	0.002

as well as *CARD8* rs2043211 (Cys10Ter) did not result significantly associated with PVL or CD4+ T cells count in this cohort after Bonferroni correction.

Taking into account that all studied SNPs affect the same complex, we then analyzed the effect of interaction (epistasis) between SNPs in different genes (*NLRP3, CARD8, P2X7, IL1B* and *IL18*) and clinical parameters by the meaning of a two-SNPs model of interaction. In Fig. 3 adjusted *p*-values for SNPs interaction log-likelihood ratio (LRT) test were graphically resumed for PVL_0 (Fig. 3a), PVL_1 (Fig. 3b), CD4+ T_0 (Fig. 3c) and CD4+ T_1 (Fig. 3d). When we performed the multivariate analysis of SNPs with major interactions score, we observed that none of the combinations significantly associated with clinical variables according to Bonferroni threshold (Supplementary File 2).

Then we tested the SNPs interaction in 10-years follow up, we observed that *NLRP3* SNPs together (rs35829419 and rs10754558) or as well as rs35829419 (Gln705Lys) and *CARD8* rs2043211 (Cys10Ter) combination positively affect CD4+ T cells levels (Fig. 3e–f).

Altogether these results point out for an opposite role of inflammasome cytokines in chronic HIV infection. For what concerns NLRP3 receptor, it appears to exert a beneficial role during chronic infection.

According to these findings we hypothesized that NLRP3/IL-18 axis

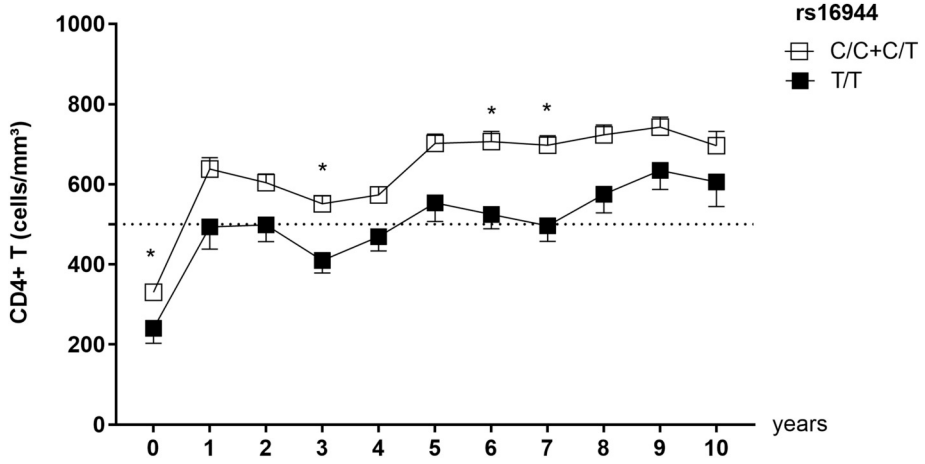

Fig. 2. Effect of variant *IL1B* rs16944 on CD4+ T lymphocytes level in HIV-infected patients. CD4+ T cells levels according to *IL1B* rs16944 genotypes during 10-years follow up. Multiple *t*-test significance (*: $p < .05$). Dotted line indicated the threshold for CD4+ T cells (500 cells/mm^3).

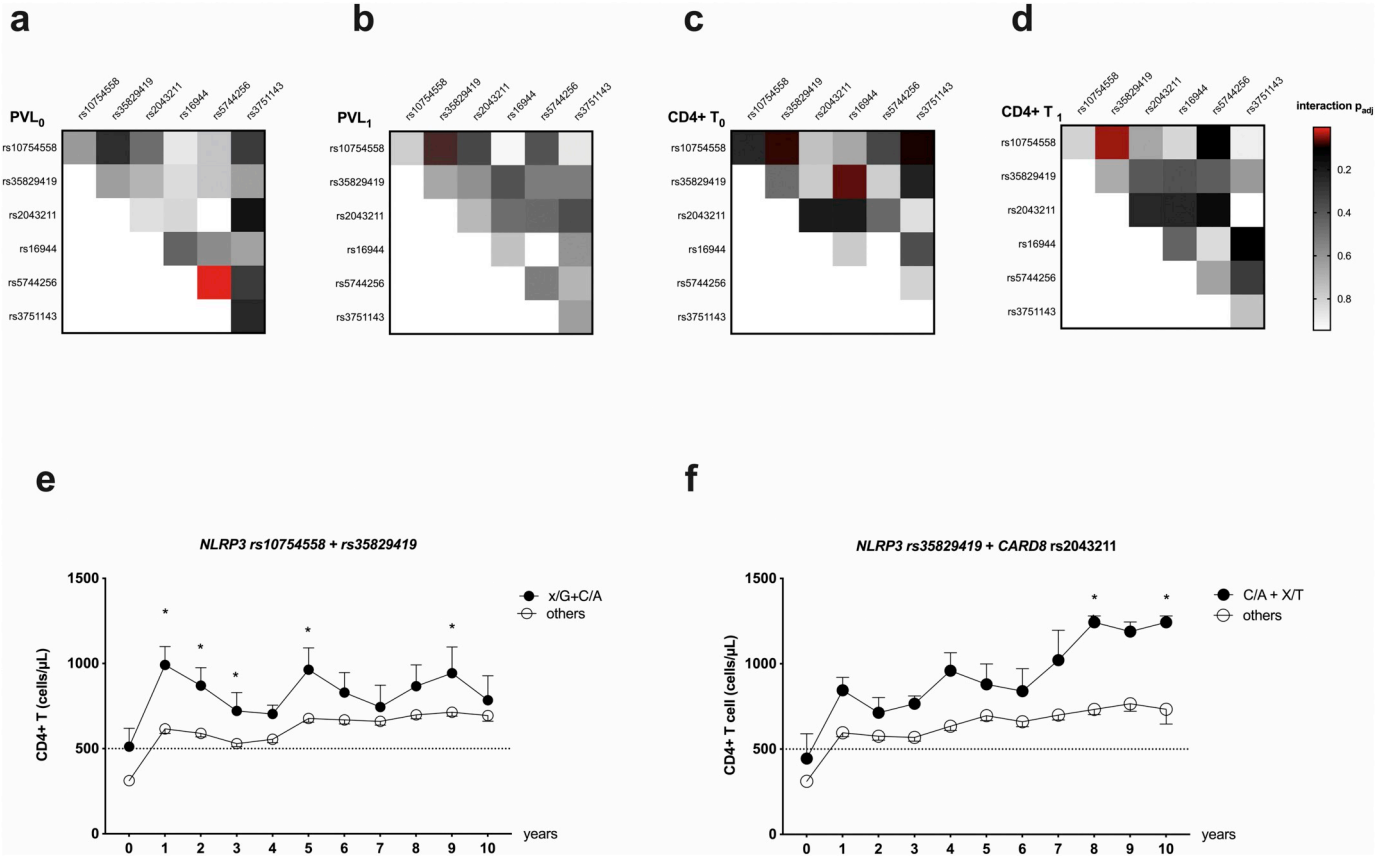

Fig. 3. Effect of SNP-SNP interaction on clinical variable of HIV-infected patients.

a–d) A SNP-SNP interaction analysis was performed in cohort 1 by the meaning of a log-likelihood ratio test. Interaction plots for each SNP using for genetic models. Each plot contains the adjusted *p*-values (p_{adj}) for the interaction (epistasis) log-likelihood ratio (LRT) test obtained for PVL_0 (a), PVL_1 (b), CD4 + T_0 (c) and CD4 + T_1 (d) variables.

e) 10-years time-course of CD4 + T cells count in HIV-infected patients of cohort 1. Data were grouped according to *NLRP3* rs10754558 C > G and rs35829419 C > A individual genotypes (dominant model for rs10754558). Multiple *t*-test significance (*: $p < .05$). Dotted line indicated the threshold for T CD4 + cells (500 cells/mm^3).

f) 10-years time-course of CD4 + T cells count in HIVinfected patients of cohort 1. Data were grouped according to *NLRP3* rs35829419 C > A and *CARD8* rs2043211 individuals genotypes (dominant model for rs2043211). Multiple t-test significance (*: $p < .05$). Dotted line indicated the threshold for T CD4 + cells (500 cells/mm^3).

could be relevant in natural infection and contribute to the HIV controlling phenotype. Therefore we genotyped the eight polymorphisms in a cohort ($n = 133$) of untreated HIV-infected patients classified in elite controllers, long-term non-progressors and progressors (cohort 2). However any statistical significant difference after Bonferroni correction was observed when SNPs frequency was compared between LTNP/EC and P (Supplementary File 3), even if a trend of protective effect toward a worse progression was observed for *NLRP3* rs10754558 ($p_{adj} = 0.034$; $OR_{adj} = 0.24$).

Finally, we have the opportunity to test the same polymorphisms in a limited ($n = 19$) but an interesting cohort of untreated HIV-infected individuals submitted to DC-based immunotherapy and followed up for one year. Previous results from our group have shown that individual genetic background could influence the immunization success in these patients [26,27] as well as it affects HIV susceptibility and/or prognosis, possibly through modulation of immune cells status. Therefore, we hypothesize to observe a similar trend of association between individuals with good control of infection in cohort 1 (ART-treated HIV patients) and individuals with a successful immunization in cohort 3 (DC-immunized HIV patients), and vice-versa. With this purpose, a multivariate analysis was realized for the SNPs according to PVL and CD4 + T lymphocytes count. Age, sex and time of disease were included in the analysis as confounding variables. Main results are presented in Table 4.

In line with the above-mentioned findings, *IL1B* rs16944 significantly associated with high PVL_0 in this cohort ($p_{adj} = 0.005$). On the other side, the loss-of-function variant rs2043211 in *CARD8* positively impact CD4 + T_0 counts ($p_{adj} = 0.009$), whereas *P2X7* rs3751143 was encountered to be more frequent in patients with a reduced CD4 + T counts before the immunization ($p_{adj} = 0.010$). The same variant appeared to be more frequent in individuals with a high PVL_1 ($p_{adj} = 0.012$).

Once again, our results pointed to an opposite role of IL-1ß and NLRP3 pathway.

Trying to better understand how inflammasome genetics could affect immunity in these patients, we then performed a multivariate analysis taking into account phenotypic and functional characterization of monocytes, DC and T lymphocytes of HIVinfected patients before the immunization. Age, sex and time of disease were included in the analysis as confounding variables. Due to the limited size of cohort, we decided to exclude results with groups of only 1 patient, independently from the *p*-value. Statistical significant results are summarized in Table 5 (complete results in Supplementary File 4) and selected results are reported graphically in Fig. 4.

As detailed below, the analysis returned a set of results that quite fitting with previous findings. Two SNPs that positively increased

Table 4
Multivariate analysis results for inflammasome SNPs association with HIV-infected patients response to dendritic cell-based immunotherapy. Multivariate analysis was performed for inflammasome SNPs and plasma viral load (PVL) and CD4 T cells count (CD4+ T) before and after dendritic cell-based immunotherapy. Sex, age and time of disease were included in the analysis as confounder variables. Only statistically significant results after correction for multiple comparisons ($p < .013$) are reported. Gene, single nucleotide polymorphism (SNP), genotypes distribution (individual count, n), mean ± standard error (SE), adjustes p-values (p_{adj}) are reported for plasma viral load before (PVL_0) and after (PVL_1) the start of ART, and for CD4+ T cells count before (CD4+ T_0) and after (CD4+ T_1) the start of immunization.

Clinical variable	Gene/SNP ID (minor allele)	Genotypes (n)	Mean ± SE	p_{adj}
Before immunotherapy				
PVL_0 (log RNA copies/mm^3)	*IL1B* rs16944 (T)	C/C (7) C/T (8) T/T (4)	3.9 ± 0.2 4.0 ± 0.2 4.8 ± 0.3	0.005
CD4 + T_0 (cells/mm^3)	*CARD8* rs2043211 (T)	A/A (7) A/T (9) T/T (3)	378.1 ± 37.7 508.9 ± 30.6 490.0 ± 26.85	0.009
	P2X7 rs3751143 (C)	A/A (12) A/C (5) C/C (2)	492.8 ± 28.2 390.8 ± 34.62 360.0 ± 40.0	0.010
After immunotherapy				
PVL_1 (log RNA copies/mm^3)	*P2X7* rs3751143 (C)	A/A (12) A/C (5) C/C (2)	2.9 ± 0.4 4.6 ± 0.4 −0.0 ± 0.2	0.012

NLRP3 inflammasome activation, namely *NLRP3* rs10754558 and *CARD8* rs2043211, associated with a better quality of monocytes-derived DC suggests that patients carrying these SNPs were less prone to constitutive immune activation than non-carriers.

On the other side, *IL1B* rs16944, which was previously associated with a worse condition of HIVinfected patients, here associated less clearly with DC activation.

Monocytes used for DC differentiation from patients with *CARD8* rs2043211 polymorphism resulted more activated (according to increased surface expression of CD86) than non-carriers ($p_{adj} = 5.5 \times 10^{-5}$) (Table 5).

iDC from patients with *NLRP3* rs10754558 or *IL1B* rs16944 variants performed a higher phagocytic activity iDC from non (Table 5; Fig. 4a–b). *NLRP3* rs10754558 oppositely affects DC-SIGN and CCR6 surface expression in iDC (Table 5).

After maturation and in vitro stimulation with autologous inactivated virus, mDC from rs10754558 carriers resulted more activated, at least in term of surface expression (Table 5; Fig. 4c). CCR5 and CCR6 resulted significantly downregulated in these patients compared to non-carriers (Table 5; Fig. 4d–e). Accordingly, the loss-of-function variant *CARD8* rs2043211 positively associated with CD86 surface expression (Table 5). *IL1B* rs16944 significantly associated with surface expression of chemokine receptor CXCR4 (Table 5; Fig. 4f).

4. Discussion

The main findings in this study including: [1] it described the distribution of NLRP3 inflammasome SNPs in ART-treated HIV-infected patients showing that a gain-of-function of NLRP3 is beneficial for clinical outcome; [2] it detected the significant and opposite association of genetic variants in *IL1B* and *IL18* with HIV+ clinical outcome; [3] it suggests that the same variants affect HIV+ clinical outcome in ART-treated patients, in DC-immunized patients as well in untreated ones, possibly due to a common effect on patients' immune cells.

Recently it has been proposed that, in ART era, chronic HIV+ infection becomes a chronic inflammatory disease [17]. As we have previously demonstrated that gain-of-function SNPs in NLRP3 inflammasome protect against HIV infection [13,14], but those same variants were associated also to sterile inflammatory diseases (such as autoimmune diseases, neurologic disorders, obesity and metabolic syndrome, etc), we questioned the role of NLRP3 inflammasome variants in clinical outcome of chronic HIV-infected patients.

The present study pointed out that in untreated HIV-infected patients a diminished activation of inflammasome (reduced level of IL-18 or loss-of-function of P2X7) is, as expected, associated with poor control of viral replication (high PVL; low CD4+ T level). However, after ART, our findings suggest a more complex scenery where genetic variations leads to increased inflammasome activation (i.e.: NLRP3 Gln705Lys + CARD8 Cys10Ter) and is still associated to PVL control, but with an unexpected effect on CD4+ T count depending on the candidate gene, such as for the gain-of-function *IL1B* rs16944 which resulted more frequent in patients with a reduced level of CD4+ T cells. These findings could be explained considering that despite the ART-induced viral suppression, HIV-infected patients continue to present chronic inflammation and immune activation, and individuals carrying pro-inflammatory polymorphisms could be benefited in terms of viral control but disadvantaged due to the amplification effect of these variants on chronic inflammation. Moreover, it is noteworthy that while an increased amount of IL-1ß appeared to be bad for chronic patients, for IL-18 it's just the opposite, reinforcing the idea of a distinct role of the two inflammasome-derived cytokines on immune system activation [1,2]. As immune activation in chronic HIV infection is mainly due to intestinal microbial translocation through a compromised gut epithelium [17], it appears that SNPs which contribute to a reduce epithelium integrity and microbiota imbalance, such as the loss-of-function variants in *IL18* promote also a worse patients' condition.

These data support the idea that an increased constitutive activation of NLRP3 inflammasome is associated with a better control of HIV-1 infection, independently of the genetic variant is a gain-of-function of inflammasome sensor NLRP3 (rs10754558, rs35829419) or a loss-of-function of the NLRP3-inhibitor CARD8 (rs2043211) or of the purinergic receptor P2X7 (rs3751143, rs2230911), which is involved in NLRP3 activation through the mediation of ATP-dependent K+ efflux.

On the other hand, it is intriguing to understand why we observed opposite results for cytokine variants. While the rs1834481 polymorphism, which negatively affects IL-18 plasma level [28], resulted more frequent in patients with higher PLV_0, and according to above-mentioned data, represent a risk factor for PVL control, the rs16944 SNP resulted associated to low CD4+ T cells counts after the ART, and high PVL_0 in naïve HIV-infected patients submitted to immunotherapy. This promoter variant (−511 T) is known to increase *IL1B* transcription [29] and could be related to a constitutive high availability of pro-IL-1ß for caspase-1 cleavage, and consequently to higher production of the pro-inflammatory cytokine. The observed association of gain-of-function *IL1B* SNP with a worse condition of HIV-infected patients appeared to be coherent with previously reported studies on chronic inflammation in these individuals [17] and also with recent findings of inflammasome activation (ASC-specks) in HIV+ PBMC [18].

We can try to explain these apparently incongruences taking into account the diverse roles of NLRP3 inflammasome in driving IL-1ß or IL-18 production in a cell-type- or signal-dependent manner, in maintaining homeostasis or even in exacerbating pathologic conditions [1,2]. Therefore, we can hypothesize that while IL-1ß represents a risk factor for chronic HIV-1 infection, possibly augmenting a positive feedback loop on immune cells, NLRP3 inflammasome activation per se contributes to a better response against the virus in the acute infection as well as in chronic condition. This protective effect could be related to an increased response of immune cells against new infection cycles (anti-viral role of NLRP3) and/or to the homeostatic role of NLRP3

Table 5
Multivariate analysis results for inflammasome SNPs association with HIV patients dendritic cells quality markers and functionality assays. Multivariate analysis was performed for inflammasome SNPs and quality markers and functionality assays of dendritic cell obtained from cohort 3 HIV-infected patients (n = 19). Sex, age and time of disease were included in the analysis as confounder variables. Only statistically significant results after correction for multiple comparisons ($p < .013$) are reported. Dendritic cells precursors (monocytes), immature (iDC) and mature dendritic cells (mDC) were examined for surface activation markers. iDC were tested also for phagocityc capacity. Gene, single nucleotide polymorphism (SNP), genotypes distribution (individual count, n), mean ± standard error (SE) for median fluorescence intensity (MFI) or percentage of fluorescent staining positive cells (%), adjustes p-values (p_{adj}) are reported.

Characteristic	Gene, SNP ID (minor allele)	Genotypes (n)	Mean ± SE	p_{adj}
		Monocytes		
CD86+ cells (MFI)	*CARD8* rs2043211 (T)	A/A (7) A/T (8) T/T (3)	2162 ± 573.0 4206 ± 1051.4 1082 ± 312.5	5.5×10^{-5}
		iDC		
Phagocytosis (MFI)	*NLRP3* rs10754558 (G)	C/C (3) C/G (4) G/G (8)	2421 ± 378.2 2135 ± 990.1 8732 ± 1553.0	0.012
	IL1B rs16944 (T)	C/C (5) C/T (7) T/T (3)	5400 ± 1368.5 3742 ± 1222.5 11,814 ± 3377.3	0.008
CCR6 + cells (%)	*NLRP3* rs10754558 (G)	C/C (3) C/G (4) G/G (8)	3.5 ± 2.1 0.4 ± 0.2 0.8 ± 0.2	0.004
DCSIGN + cells (MFI)	*NLRP3* rs10754558 (G)	C/C (3) C/G (4) G/G (8)	631.0 ± 362.7 640.0 ± 202.5 1987.4 ± 248.5	0.003
		mDC		
CD86+ cells (MFI)	*CARD8* rs2043211 (T)	A/A (7) A/T (8) T/T (3)	40,410 ± 10,213 39,856 ± 9582 78,922 ± 2200	0.003
CD40+ cells (%)	*NLRP3* rs10754558 (G)	C/C (3) C/G (4) G/G (8)	94.8 ± 2.2 51.1 ± 6.2 74.5 ± 4.5	0.006
CCR5+ cells (MFI)	*NLRP3* rs10754558 (G)	C/C (3) C/G (4) G/G (8)	977.3 ± 350.2 508.8 ± 76.6 547.8 ± 109.6	0.005
CCR6+ cells (%)	*NLRP3* rs10754558 (G)	C/C (3) C/G (4) G/G (8)	10.8 ± 4.1 1.7 ± 1.2 0.8 ± 0.1	0.001
CXCR4+ cells (MFI)	*IL1B* rs16944 (T)	C/C (5) C/T (7) T/T (3)	789.4 ± 172.6 1743.0 ± 195.9 2256.0 ± 475.6	9.1×10^{-5}

within mucosal surfaces through the induction of IL-18 [1,2]. According to this concept, HIV-infected individuals carrying gain-of-function variants of NLRP3 inflammasome, and/or in *IL18* gene, could be more protected than non-carriers against gut damage and microbial translocation, and the consequent systemic activation and exhaustion of immune cells.

We are aware that the study presents some limitations, such as the limited size of cohort 2 and 3, however these two cohorts are very original and poor exploited by genetics approach, therefore we believe that their investigation confers originality to the entire study.

5. Conclusion

NLRP3 inflammasome genetics contribute to clinical outcome of HIV infection in treated as well as in untreated patients, due to its role on activation state of peripheral immune cells.

Funding

This work was supported by the São Paulo Research foundation (FAPESP) (2013/06142-1; 2015/23395-6; 2015/50650-7), E.C.R. and V.N.C.L. are recipient of a PhD Fellowship from FAPESP. A.P. is recipient of a Fellowship from CNPq.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Acknowledgements

We thank the SEAPH (Department of Infectious Disease, Faculty of Medicine, University of São Paulo, Brazil) for SP HIV-infected patients' recruitment; Dr. Alexandre De Almeida (Faculty of Medicine, University of São Paulo, Brazil) for Immunized HIV-infected patients' recruitment. We wish to thank all patients for the collaboration.

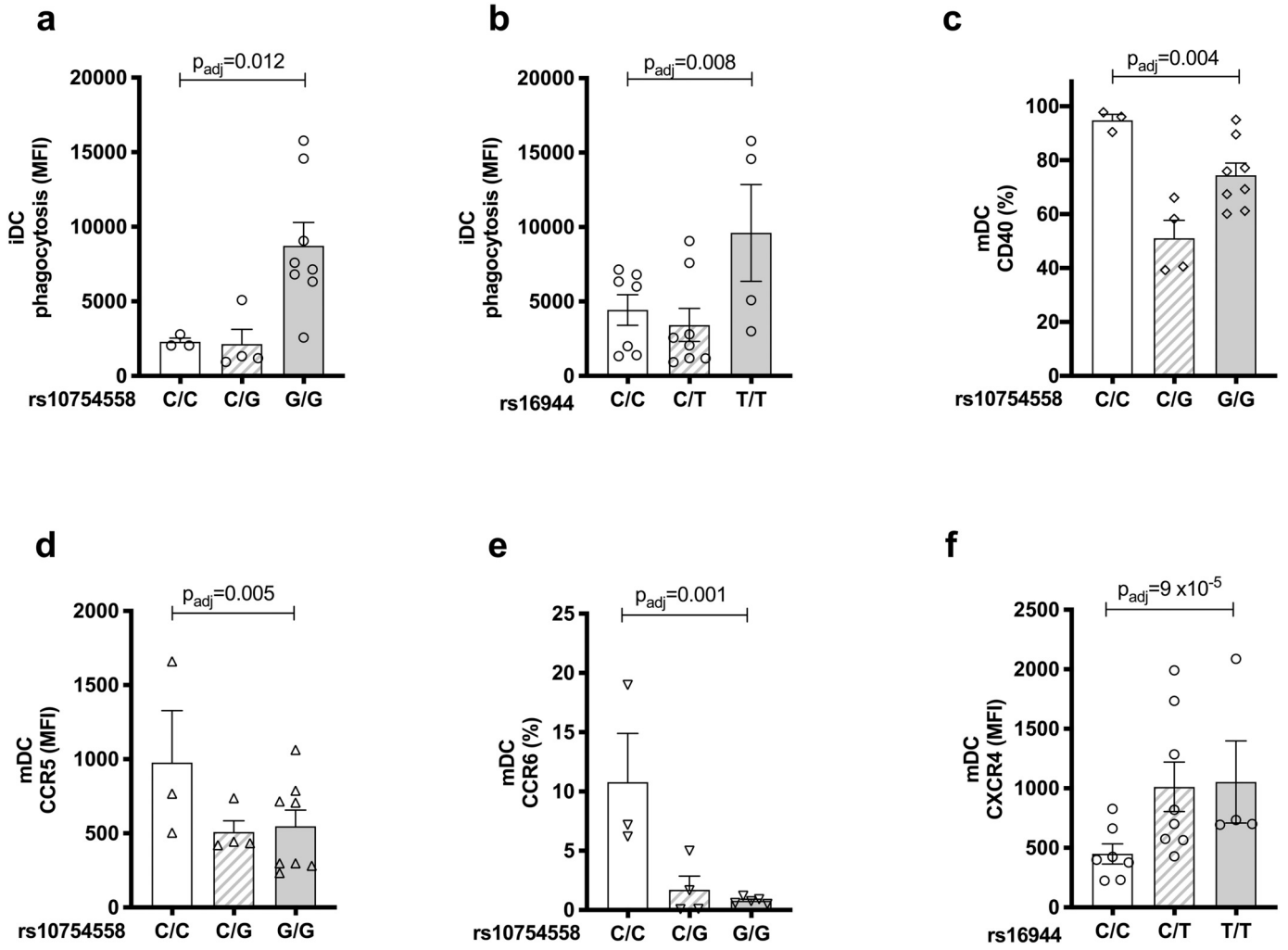

Fig. 4. Effect of inflammasome SNPs on dendritic cells quality.
DC donors were genotyped for *NLRP3* rs10754558 and *IL1B* rs16944 SNPs and functional cell data were grouped accordingly.
a–b) 1×10^6 iDC were incubated with 1 mg/ml fluorescently-labelled (FITC)-dextran for 60 min at 37 °C 5% CO_2, before cell surface staining with anti-CD14 and anti-CD11c fluorescently-labelled antibodies for 20 min at 4 °C. Phagocytosis capacity of iDC was evaluated by cell internalization of FITC-dextran and flow cytometry (mean fluorescence intensity, MFI). At least 50,000 events were acquired by a LRS Fortessa flow cytometry equipment (BD Bioscience). Data were grouped according to rs10754558 C > G (a) and rs16944 C > T (b) genotypes.
c-f) 0.2×10^6 mDC were stained with anti-CD40, anti-CCR5, anti- CCR6 and anti-CXCR4 fluorescently-labelled antibodies for 20 min at 4 °C. mDC activation state and quality was evaluated by surface markers expression levels and flow cytometry (MFI and percentage of positive cells). At least 50,000 events were acquired by a LRS Fortessa flow cytometry equipment (BD Bioscience). Data were grouped according to rs10754558 C > G (c-e) and rs16944 C > T (f) genotypes.
Mean values and standard errors are indicated. Multivariate analysis adjusted *p*-values (p_{adj}) are shown. MFI: median fluorescence intensity; %: percentage of positive cells.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2019.108266.

References

[1] M. Lamkanfi, V.M. Dixit, Mechanisms and functions of inflammasomes, Cell 157 (2014) 1013–1022.
[2] M.N. Patel, R.G. Carroll, S. Galvan-Pena, E.L. Mills, R. Olden, M. Triantafilou, A.I. Wolf, C.E. Bryant, K. Triantafilou, S.L. Masters, Inflammasome priming in sterile inflammatory disease, Trends Mol. Med. 23 (2017) 165–180.
[3] D. Verma, E. Sarndahl, H. Andersson, P. Eriksson, M. Fredrikson, J.I. Jonsson, M. Lerm, P. Soderkvist, The Q705K polymorphism in NLRP3 is a gain-of-function alteration leading to excessive interleukin-1beta and IL-18 production, PLoS One 7 (2012) e34977.
[4] Y. Hitomi, M. Ebisawa, M. Tomikawa, T. Imai, T. Komata, T. Hirota, M. Harada, M. Sakashita, Y. Suzuki, N. Shimojo, Y. Kohno, K. Fujita, A. Miyatake, S. Doi, T. Enomoto, M. Taniguchi, N. Higashi, Y. Nakamura, M. Tamari, Associations of functional NLRP3 polymorphisms with susceptibility to food-induced anaphylaxis and aspirin-induced asthma, J. Allergy Clin. Immunol. 124 (2009) 779–785 e776.
[5] S. Ito, Y. Hara, T. Kubota, CARD8 is a negative regulator for NLRP3 inflammasome, but mutant NLRP3 in cryopyrin-associated periodic syndromes escapes the restriction, Arthritis Res. Ther. 16 (2014) R52.
[6] F. Di Virgilio, D. Dal Ben, A.C. Sarti, A.L. Giuliani, S. Falzoni, The P2X7 receptor in infection and inflammation, Immunity 47 (2017) 15–31.
[7] C.M. Miller, N.R. Boulter, S.J. Fuller, A.M. Zakrzewski, M.P. Lees, B.M. Saunders, J.S. Wiley, N.C. Smith, The role of the $P2X_7$ receptor in infectious diseases, PLoS Pathog. 7 (2011) e1002212.
[8] G.V. Paramel, A. Sirsjo, K. Fransen, Role of genetic alterations in the NLRP3 and CARD8 genes in health and disease, Mediat. Inflamm. 2015 (2015) 846782.
[9] D. Souza de Lima, M.M. Ogusku, A. Sadahiro, A. Pontillo, Inflammasome genetics contributes to the development and control of active pulmonary tuberculosis, Infect. Genet. Evol. 41 (2016) 240–244.
[10] S.L. Fernando, B.M. Saunders, R. Sluyter, K.K. Skarratt, J.S. Wiley, W.J. Britton, Gene dosage determines the negative effects of polymorphic alleles of the P2X7 receptor on adenosine triphosphate-mediated killing of mycobacteria by human macrophages, J. Infect. Dis. 192 (2005) 149–155.
[11] A.N. Shemon, R. Sluyter, S.L. Fernando, A.L. Clarke, L.P. Dao-Ung, K.K. Skarratt, B.M. Saunders, K.S. Tan, B.J. Gu, S.J. Fuller, W.J. Britton, S. Petrou, J.S. Wiley, A Thr357 to Ser polymorphism in homozygous and compound heterozygous subjects causes absent or reduced P2X7 function and impairs ATP-induced mycobacterial

killing by macrophages, J. Biol. Chem. 281 (2006) 2079–2086.
[12] C.A. Yang, B.L. Chiang, Inflammasomes and human autoimmunity: a comprehensive review, J. Autoimmun. 61 (2015) 1–8.
[13] A. Pontillo, L.A. Brandao, R.L. Guimaraes, L. Segat, E. Athanasakis, S. Crovella, A 3'UTR SNP in NLRP3 gene is associated with susceptibility to HIV-1 infection, J. Acquir. Immune Defic. Syndr. 54 (2010) (1999) 236–240.
[14] A. Pontillo, T.M. Oshiro, M. Girardelli, A.J. Kamada, S. Crovella, A.J. Duarte, Polymorphisms in inflammasome' genes and susceptibility to HIV-1 infection, J. Acquir. Immune Defic. Syndr. 59 (2012) (1999) 121–125.
[15] H. Guo, J. Gao, D.J. Taxman, J.P. Ting, L. Su, HIV-1 infection induces interleukin-1beta production via TLR8 protein-dependent and NLRP3 inflammasome mechanisms in human monocytes, J. Biol. Chem. 289 (2014) 21716–21726.
[16] A. Pontillo, L.T. Silva, T.M. Oshiro, C. Finazzo, S. Crovella, A.J. Duarte, HIV-1 induces NALP3-inflammasome expression and interleukin-1beta secretion in dendritic cells from healthy individuals but not from HIV-positive patients, AIDS (London, England) 26 (2012) 11–18.
[17] S.G. Deeks, S.R. Lewin, D.V. Havlir, The end of AIDS: HIV infection as a chronic disease, Lancet (London, England) 382 (2013) 1525–1533.
[18] A. Bandera, M. Masetti, M. Fabbiani, M. Biasin, A. Muscatello, N. Squillace, M. Clerici, A. Gori, D. Trabattoni, The NLRP3 Inflammasome is upregulated in HIV-infected antiretroviral therapy-treated individuals with defective immune recovery, Front. Immunol. 9 (2018) 214.
[19] F. Ahmad, N. Mishra, G. Ahrenstorf, B.S. Franklin, E. Latz, R.E. Schmidt, L. Bossaller, Evidence of inflammasome activation and formation of monocyte-derived ASC specks in HIV-1 positive patients, AIDS (London, England) 32 (2018) 299–307.
[20] H. Guo, J.B. Callaway, J.P. Ting, Inflammasomes: mechanism of action, role in disease, and therapeutics, Nat. Med. 21 (2015) 677–687.
[21] B.-Z. Shao, Z.-Q. Xu, B.-Z. Han, D.-F. Su, C. Liu, NLRP3 inflammasome and its inhibitors: a review, Front. Pharmacol. 6 (2015) 262.
[22] T.C. Ferreira, E.P. Sampaio, G.A. Arganaraz, M.V. Gondim, L. Shapiro, E.R. Arganaraz, Increased prevalence of the alpha-1-antitrypsin (A1AT) deficiency-related S gene in patients infected with human immunodeficiency virus type 1, J. Med. Virol. 86 (2014) 23–29.
[23] W. Lu, L.C. Arraes, W.T. Ferreira, J.M. Andrieu, Therapeutic dendritic-cell vaccine for chronic HIV-1 infection, Nat. Med. 10 (2004) 1359–1365.
[24] H. Akaike, A new look at the statistical model identification, IEEE Trans. Autom. Control 19 (1974) 716–723.
[25] J.C. Barrett, Haploview: visualization and analysis of SNP genotype data, Cold Spring Harb Protoc 2009 (2009) pdb.ip71.
[26] R. Moura, A. Pontillo, P. D'Adamo, N. Pirastu, A. Campos Coelho, S. Crovella, Exome analysis of HIV patients submitted to dendritic cells therapeutic vaccine reveals an association of CNOT1 gene with response to the treatment, J. Int. AIDS Soc. 17 (2014) 18938.
[27] E.C. Reis, L.T. da Silva, W.C. da Silva, A. Rios, A.J. Duarte, T.M. Oshiro, S. Crovella, A. Pontillo, Host genetics contributes to the effectiveness of dendritic cell-based HIV immunotherapy, Hum. Vaccines Immunotherapeutics 14 (2018) 1995–2002.
[28] S. Martinez-Hervas, V. Martinez-Barquero, E. Nunez Savall, V. Lendinez, L. Olivares, E. Benito, J.T. Real, F.J. Chaves, J.F. Ascaso, [Plasma IL-18 levels are related to insulin and are modulated by IL-18 gene polymorphisms], Clinica e investigacion en arteriosclerosis: publicacion oficial de la Sociedad Espanola de, Arteriosclerosis 27 (2015) 265–271.
[29] S.K. Hall, D.G. Perregaux, C.A. Gabel, T. Woodworth, L.K. Durham, T.W. Huizinga, F.C. Breedveld, A.B. Seymour, Correlation of polymorphic variation in the promoter region of the interleukin-1 beta gene with secretion of interleukin-1 beta protein, Arthritis Rheum. 50 (2004) 1976–1983.