HIV-1 induces NALP3-inflammasome expression and interleukin-1β secretion in dendritic cells from healthy individuals but not from HIV-positive patients

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Objective: NALP3-inflammasome is an innate mechanism, alternative to type-1 interferon, which is able to recognize nucleic acids and viruses in the cytoplasm and to induce pro-inflammatory response. Here, we hypothesized the involvement of inflammasome in the early defense against HIV-1 and in the full maturation of dendritic cells: for this, we evaluated the response of dendritic cells pulsed with HIV-1 in terms of inflammasome activation in healthy donors. Moreover, inflammasome response to HIV was evaluated in HIV-infected individuals.

Design and methods: Monocyte-derived dendritic cells isolated from 20 healthy individuals (HC-DC) and 20 HIV-1-infected patients (HIV-DC) were pulsed with alditrithiol-2-inactivated HIV-1. We then analyzed inflammasome genes expression and interleukin-1β (IL-1β) secretion.

Results: In HC-DC, HIV-1 induced higher *NLRP3/NALP3* mRNA expression compared with other inflammasome genes such as *NALP1/NLRP1* or *IPAF/NLRC4* ($P < 0.001$). This augmented expression was accompanied by *CASP1*-increased and *IL1B*-increased mRNA levels and by a significant increment of IL-1β secretion ($P < 0.05$). Otherwise, HIV-1 failed to activate inflammasome and cytokine production in HIV-DC. HIV-DC showed an increased *NLRP3/NALP3* basal expression, suggesting a chronic inflammatory profile of patients' immune cells.

Conclusion: HIV-1 was able to induce a NALP3-inflammasome response in healthy individuals, indicating that this inflammasome could play a role in the first steps of HIV-1 infection; the consequent inflammatory process may be important for directing host immune response against the virus and/or disease progression. HIV-DC seemed to be chronically activated, but unresponsive against pathogens. Our findings could be of interest considering the ongoing research about dendritic cell manipulation and therapeutic strategies for AIDS involving dendritic cell-based immune-vaccines.

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Keywords: dendritic cells, HIV-1, immunotherapy, inflammasome, interleukin-1β, NALP3, vaccine

Introduction

In the last 10 years, an increasing number of dendritic cell-based therapeutic vaccination trials against HIV-1 have been developed, augmenting the general interest in dendritic cell biology and in the interaction between this innate immune cell and HIV-1 [1].

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Innate immune recognition plays a central role in immune activation against HIV-1 through pattern recognition receptors (PRRs) activation. PRRs are involved in recognition processes (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin/DC-SIGN, Toll-like receptors/TLR7/8), hence resulting in the activation of pro-inflammatory transcription factor nuclear factor-κB and in the production of a range of cytokines and chemokines. This innate immune system activation leads to an antiviral response including not only the adaptive system but also activates HIV-1 replication in cells with established infection, leading to a chronic infection and immune activation [1–3]. HIV-1 infection possibly begins with the contact of the virus with mucosal or intraepithelial dendritic cells, being the first responsible of the HIV-1-associated immune response [1].

Three cytoplasmic members of PRR family, namely NALP1, NALP3 and IPAF, are capable to sense several – and often distinct – microbial and particulate molecules within the cell and to activate the molecular platform known as inflammasome and consequent caspase-1-dependent secretion of interleukin-1β (IL-1β) [4].

Several viruses are able to activate caspase-1 and IL-1β, evidencing the potential role of inflammasomes in the immune response to viruses [5].

Particularly, NALP3 inflammasome and IL-1β have been reported to be an innate mechanism alternative to the type I interferon, which are able to recognize nucleic acids (DNA or RNA) and cytoplasmatic viral particles (influenza virus, Sendai virus, adenovirus) and to trigger a pro-inflammatory response [4]. Similarly, it could be hypothesized that NALP3 acts in dendritic cell sensing HIV-1, driving the early activation of immune system against the virus.

Furthermore, it has been reported that NALP3 inflammasome is involved in the full activation of dendritic cell by common vaccine adjuvant or particulate molecules and in the specific induction of T helper/Th2 response [4]. Thus, inflammasome may play an important role not only in sensing HIV-1 and to elicit an inflammatory response, but also in the activation of acquired immunity against the virus. We can, therefore, speculate that the amount of inflammasome activation and IL-1β secretion could in someway modulate the immune response against HIV-1, helping the virus elimination or, on the contrary, the virus-induced loss of immune cells.

Mutations in *NALP3/NLRP3* are responsible for three rare hereditary periodic fevers, familial cold urticaria (MIM_120100), Muckle–Wells syndrome (MIM_191900) and chronic infantile neurologic cutaneous arthicular syndrome (MIM_ 607115); the identified nucleotide variations are mainly gain of function mutations leading to an increased activation of the inflammasome, resulting in dramatic high production of IL-1β [4]. Polymorphisms in NALP3/*NLRP3* gene have been associated with increased susceptibility to infectious agents such as HIV-1 [6] and Candida [7], and to the development of autoimmune diseases such as type-1 diabetes and celiac disease [8], reinforcing the idea that a deregulation in inflammasome and in the IL-1β secretion could lead to an impaired immune response.

Starting from these elements, we hypothesized that HIV-1 could stimulate NALP3-inflammasome in dendritic cell, driving immune system activation against the virus. Dendritic cell response to HIV through NALP3-inflammasome could be important in HIV vaccine development [9], especially in dendritic cell-based immune treatment [10].

In our study, we evaluated HIV-induced expression of the inflammasome receptors, NALP3/*NLRP3*, NALP1/*NLRP1* and IPAF/*NLRC4*, and of the two effector molecules caspase-1/*CASP1* and IL-1β/*IL1B* in dendritic cells from healthy individuals and HIV-positive patients; finally, IL-1β secretion was analyzed for measuring inflammasome activation.

Materials and methods

Participants

We enrolled 20 healthy individuals (eight males, 12 females; mean age 30 ± 5 SD) and 20 HIV-1-positive volunteers (15 males, five females; mean age 40.05 ± 10.88 SD): the two groups were age-balanced and ethnicity matched. HIV-positive patients were recruited from the ambulatory of secondary immunodeficiencies (ADEE 3002) of Hospital das Clinicas (Faculty of Medicine, University of Sao Paulo, Brazil). Inclusion criteria were as follows: age of at least 18 years, absence of pregnancy, HIV-1 seropositivity for at least 1 year, no clinical AIDS, hemoglobin at least 10 g/dl, platelets of at least 100 000 cells/μl, blood $CD4^+$ cells of at least 300 cells/μl, plasma viral RNA of at least 10 000 copies/ml and absence of other chronic diseases. Fourteen HIV-positive patients were in antiretroviral therapy (ART) at the moment of recruitment and six were 'naive' for treatment.

Written informed consent was obtained according to the protocol of 'Hospital das Clinicas' Ethical Committee (CAPPesq) (number 0791/09, 04 November 2009) (Sao Paulo, Brazil).

HIV-1 culture and virus isolation

A concentration of 20×10^6 peripheral blood mononuclear cells (PBMCs) was isolated from 10 HIV-positive

individuals by Ficoll-Paque gradient (GE Healthcare/Amersham Biosciences, Piscataway, New Jersey, USA) and cultivated in RPMI-1640 medium (Invitrogen Life Technology, Carlsbad, California, USA) with 10% Fetal Calf Serum/FCS (Invitrogen) in the presence of 20-IU/ml IL-2. Every 7 days, 20×10^6 PBMCs isolated from healthy donors, and previously activated with 20-IU/ml IL-2 and 5-μl/ml phytohemagglutinin (Gibco/Life Technology) for 48 h, were added to the virus cultures. Culture HIV positivity was measured by ELISA test for HIV-1 p24 detection (Vironostika, Biomerieux, Boxtel, Holland). Culture supernatants were collected and stored at -80°C until virus isolation. About 500 ml of cells HIV-1-positive supernatant of each culture was inactivated with 0.11-g/ml alditrithiol-2 (AT-2; Sigma-Aldrich, St. Louis, Missouri, USA) and concentrated through Amicon Millipore filters (100 kDa) (Millipore, Billerica, Massachusetts, USA). AT-2-inactivated virus (AT-HIV) was finally ultracentrifuged on a 20% sucrose-Tris NaCl EDTA/TNE solution (20 mmol/l Tris/HCl, 20 mmol/l NaCl, 2.5 mmol/l EDTA; Sigma-Aldrich) and isolated as described elsewhere [10]. A pool of the 10 AT-1-inactivated viruses (eight out of 10 are HIV subtype B, two is HIV subtype E), collected from 10 HIV-positive patients distinct from the individuals analyzed in our study, was prepared and used in all the experiments.

Monocyte-derived immature dendritic cells

Monocytes were isolated by adherence from PBMCs obtained by centrifugation over Ficoll-Paque gradient and cultured at $1.5-2 \times 10^6$ cells/ml in AIM-V medium (Gibco) containing 50 ng/ml granulocyte-macrophage colony-stimulating factor (GM-CSF) (Peprotech, Rocky Hill, New Jersey, USA) and 50 ng/ml IL-4 (Peprotech). On day 3, one-half of the medium was replaced by fresh AIM-V with GM-CSF and IL-4. On day 6, monocytes-derived dendritic cells were pulsed with 0.5×10^9 particles of AT-HIV or 1 μg/ml lipopolysaccharide (LPS; Sigma-Aldrich). Then supernatants were harvested and used for IL-1β secretion analysis and the cells were lysed for mRNA isolation. In some experiments, AT-HIV-pulsed dendritic cell were treated with the 'maturation' cocktail [50 ng/ml IL-4, 50 ng/ml GM-CSF, 50 ng/ml tumor necrosis factor (TNF), 10 ng/ml IL-1β, 100 ng/ml IL-6] for 48 h to obtain mature dendritic cell [10].

Dendritic cells have been also treated with AT-2 (0.11 g/ml) alone in order to text the contribution of this chemical to our findings.

Flow cytometry

Fluorochrome-conjugated monoclonal antibodies (BD Biosciences, Franklin Lakes, New Jersey, USA) were used for Fluorescence-activated cell sorting (FACS) analysis. Cells were fixed in 2% paraformaldehyde before acquisition on a BD FACSCalibur flow cytometer.

RNA isolation and real time-PCR

Total RNA was isolated using the RNAqueous micro kit (Ambion, Life Technologies) and retrotranscribed with the SuperScritp-II kit (Invitrogen). *NLRP1, NLRP3, NLRC4, CASP1* and *IL1B* genes were amplified with specific TaqMan Gene Expression Assays (Applied Biosystems/Life Technologies) using the ABI 7300 SDS platform (Applied Biosystems/Life Technologies). *ACTB* was the housekeeping gene used for normalization. Relative quantitative expression was calculated as $2\text{exp}-\Delta\Delta\text{Ct}$ following the indications by Livak and Schmittgen [11].

Interleukin-1β measurement

The secreted IL-1β was evaluated with ELISA (IL-1β assay, BD Biosciences). Results were expressed as picograms per milliliter.

Statistical analysis

One-way analysis of variance (ANOVA) analysis was used and a significance level of 0.05 was applied. Tukey's multiple comparison test was applied as posttest to compare different groups. Prism 5.01 software (GraphPad Software Inc., La Jolla, California, USA) was used for done graphs and statistical analysis.

Results

We evaluated the expression of *NLRP1/NALP1, NLRP3/NALP3, NLRC4/IPAF, CASP1* and *IL1B* genes and the IL-1β secretion in dendritic cells from 20 healthy individuals (HC-DC) and 20 HIV-positive patients (HIV-DC) pulsed with AT-HIV for 1, 2, 4, 8, 12, 16 and 24 h; we used LPS as positive control for inflammasome activation [4].

Cells differentiation state was checked by flow cytometry looking at granulosity (Fig. 1a) and common dendritic cell markers [CD11c, human leukocyte antigen (HLA)-DR] (Fig. 1b). No significant differences were observed in differentiation markers between HC-DC and HIV-DC (HLA-DR: 98% positive cells ± 2 SD versus 97% ± 2.5; CD11c: 96.8% ± 1.8 versus 95% ± 2.9; $P > 0.05$).

In HC-DC, AT-HIV induced an important *NLRP3/NALP3* expression within 4 h (23.16-fold with respect to untreated cells) (Fig. 2a); this *NLRP3/NALP3* mRNA expression was significantly higher when compared with other inflammasome key molecules, such as *NLRP1/NALP1* ($P < 0.001$) or *NLRC4/IPAF* ($P < 0.001$) (Fig. 2a). Elevated levels of *NLRP3/NALP3* mRNA appeared within 4 h from viral exposure and then dramatically decreased (Fig. 2a).

CASP1 and *IL1B* mRNA levels reached the maximum after 2 h of AT-HIV stimulation (Fig. 2b).

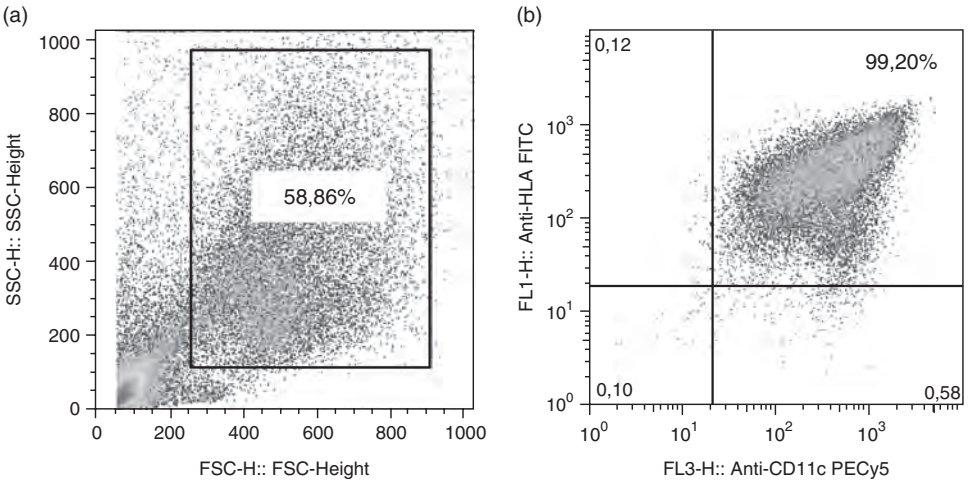

Fig. 1. Characterization of monocyte-derived immature dendritic cells. Generated immature monocyte-derived dendritic cells (DC) from healthy controls (HC-DC) and HIV-infected patients (HIV-DC) at 6 days were analyzed for basic characteristic of size and granulosity (a) and differentiation markers CD11c and HLA-DR expression (b). The analysis of one HC-DC experiment is shown as representative of all the experiments. In this sample, 58.86% are DCs. HLA-DR, human leukocyte antigen.

AT-HIV was able to induce *NLRP3, CASP1* and *IL1B* expression in HC-DC with a time course very close to those obtained with bacterial LPS (Fig. 2c).

When the same experimental protocol was applied on dendritic cells derived from HIV-positive patients, surprisingly AT-HIV was not able to significantly augment the expression levels of any of the five genes analyzed (Fig. 3a–b).

Particularly, at 4 h, the *NLRP3* expression level was very low in HIV-DC pulsed with AT-HIV when compared with HC-DC ($P < 0.001$) (Fig. 3c).

Interestingly in HIV-DC, LPS did not activate inflammasome expression too (Fig. 3d). LPS induced a significantly higher mRNA expression of *NLRP3* and *IL1B* in HC-DC than in HIV-DC ($P < 0.05$ and $P < 0.001$, respectively).

Basal *NLRP3* expression level was found almost three times increased in resting HIV-DC with respect to resting HC-DC (Fig. 4).

This unresponsive condition of HIV-DC did not correlate with the presence of ART (Supplementary Figure S1, http://links.lww.com/QAD/A185): AT-HIV induced a similar expression of inflammasome genes in naive ART HIV-DC ($P = 0.533$), whereas the expression between HC-DC and naive HIV-DC or ART HIV-DC continued to be statistically different ($P = 0.0006$ and $P < 0.0001$, respectively).

Furthermore the treatment of AT-HIV-pulsed dendritic cell with the 'maturation' cocktail (10 ng/ml IL-1β, 50 ng/ml TNF, 100 ng/ml IL-6, 50 ng/ml GM-CSF and IL-4 50 ng/ml) did not influence inflammasome expression levels.

These differences in inflammasome genes expression between HC-DC and HIV-DC are once more underlined by the results obtained analyzing IL-1β secretion in dendritic cell supernatants as marker of inflammasome activation. In Fig. 5, IL-1β secretion from HC-DC and HIV-DC after 24 h of AT-HIV or LPS stimulation is reported: shorter exposure did not result in detectable cytokine production. The remarkable increment of *NLRP3*, *CASP1* and *IL1B* genes expression levels coexisted with a sustained secretion of the cytokine in HC-DC pulsed with AT-HIV (250.45 ± 34.82 pg/ml) with respect to resting HC-DC (74.87 ± 39.09; $P < 0.001$).

Otherwise, AT-HIV (129.33 ± 78.14 pg/ml) did not induce a significant IL-1β secretion in HIV-DC when compared with resting condition (131.78 ± 83.80 pg/ml; $P > 0.05$). Also LPS was not able to increase IL-1β secretion in HIV-DC (143.69 ± 97.44 versus 131.78 ± 83.80 pg/ml; $P > 0.05$). (Fig. 5) The ART did not affect IL-1β secretion in HIV-DC (naive versus ART: $P = 0.331$) as well as inflammasome genes expression.

It is noteworthy that the cytokine secretion in resting HIV-DC (131.78 ± 83.80 pg/ml) was higher than that in resting HC-DC (74.87 ± 39.09 pg/ml; $P < 0.05$). LPS and HIV induced IL-1β secretion that was found significantly diminished in HIV-DC when compared with HC-DC ($P < 0.05$) (Fig. 5).

When treating HC-DC and HIV-DC with AT-2 alone, we did not find any difference in inflammasome genes expression levels and in IL-1β secretion compared with untreated cells (data not shown).

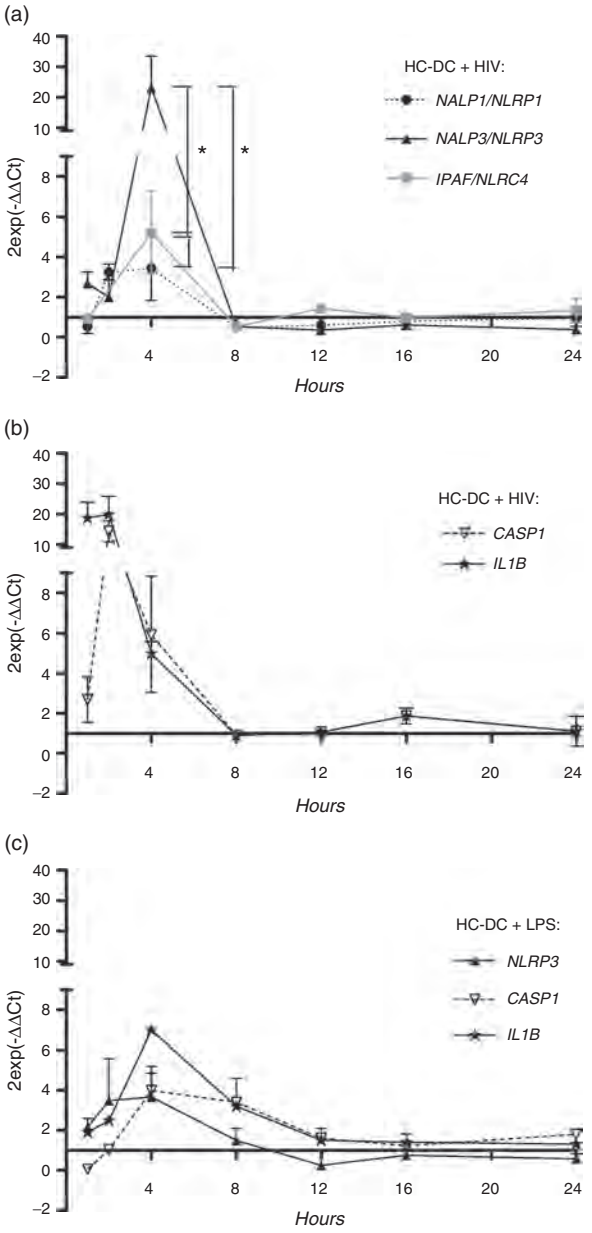

Fig. 2. Inflammasome genes expression in dendritic cells from healthy individuals pulsed with alditrithiol-2-inactivated HIV. Time course of mRNA expression at 1, 2, 4, 8, 12, 16, and 24 h after stimulation of dendritic cells of healthy individuals (HC-DC) with alditrithiol-2-inactivated HIV (AT-HIV) was reported for *NLRP1, NLRP3* and *NLRC4* (a) and *CASP1* and *IL1B* genes (b). Time course of mRNA expression at 1, 2, 4, 8, 12, 16 and 24 h after stimulation of HC-DC with lipopolysaccharide (LPS) was reported for *NLRP3*, *CASP1* and *IL1B* (c). Induced mRNA expression of each gene was calculated with respect to the unstimulated condition (*r*) as 2exp (-ΔΔCt) in which $\Delta\Delta Ct = \Delta Ct\ HIV_{HC\text{-}DC}$ or $\Delta Ct\ LPS_{HC\text{-}DC} - \Delta Ct\ R_{HC\text{-}DC}$ (resting). Results are expressed as the mean of 2exp (-ΔΔCt) ± SEM, $n = 20$. All the Ct values were normalized against *ACTB* Ct (ΔCt). One-way ANOVA analysis and Tukey's posttest were used to compare *NLRP1/NALP1, NLRP3/NALP3* and *NLRC4/IPAF* expression. $^{**}P < 0.001$. ANOVA, analysis of variance.

Discussion

Among inflammasome-forming nod-like receptors/NLRs, NALP3 has been recently proposed as a novel cytoplasmic sensor for several viruses [4,5], although no information is still available about its role in recognizing HIV-1. In our opinion, the involvement of inflammasome could be an intriguing issue considering that macrophages and dendritic cell, both target of HIV, express it, and also considering the ongoing research about dendritic cell-based treatment for HIV-infected patients.

Our results show that AT-HIV is able to induce a specific expression of NALP3-inflammasome components in dendritic cell within the early moment (up to 4 h) of virus–cell interaction. The relevant IL-1β secretion is indicative of caspase-1 activation and indirectly of inflammasome assembly, being this molecular complex responsible for the cleavage of pro-caspase-1 to caspase-1 [4].

In our cellular model, the direct activation of inflammasome could not be easily measured: transfection with recombinant proteins would be limited in primary cells, especially from HIV-infected individuals; the NALP knockout mice implied for other kind of host/pathogen studies [4,5] are not useful to mimic HIV infection and AIDS pathogenesis, as mouse is not a natural host of HIV.

Also considering this limitation, we can hypothesize that in dendritic cells, NALP3-inflammasome could play a role in first steps of HIV infection, possibly sensing viral dsRNA after virus internalization in dendritic cells through its leucine reach repeat/LRR domain, or indirectly through a downstream effect caused by the virus, as proposed elsewhere [4,5]. The consequent inflammasome activation leads to increased secretion of IL1β from these cells that could be an important microenvironmental factor capable of influencing the immune response against HIV *per se* [1,2], or considering the discovered role for inflammasome in dendritic cells [4], it could participate to full dendritic cell activation and to increased proliferation of specific HIV-induced T cells.

Our results are concordant with previously published findings reporting the exclusive activation of NALP3-inflammasome by viral particle [4,5], and also with NALP3 playing a key role in dendritic cell biology [4]. Furthermore, the importance of innate immunity in HIV-1 pathogenesis has been once more underlined [2]. The elevated secretion of IL-1β from HIV-pulsed dendritic cell resembles the high levels of TNF, IL-6 and IL-1β observed in patients' plasma and lymph nodes during early stage of HIV infection [3].

Despite several intracellular restriction factors for HIV replication have been described in immune cells

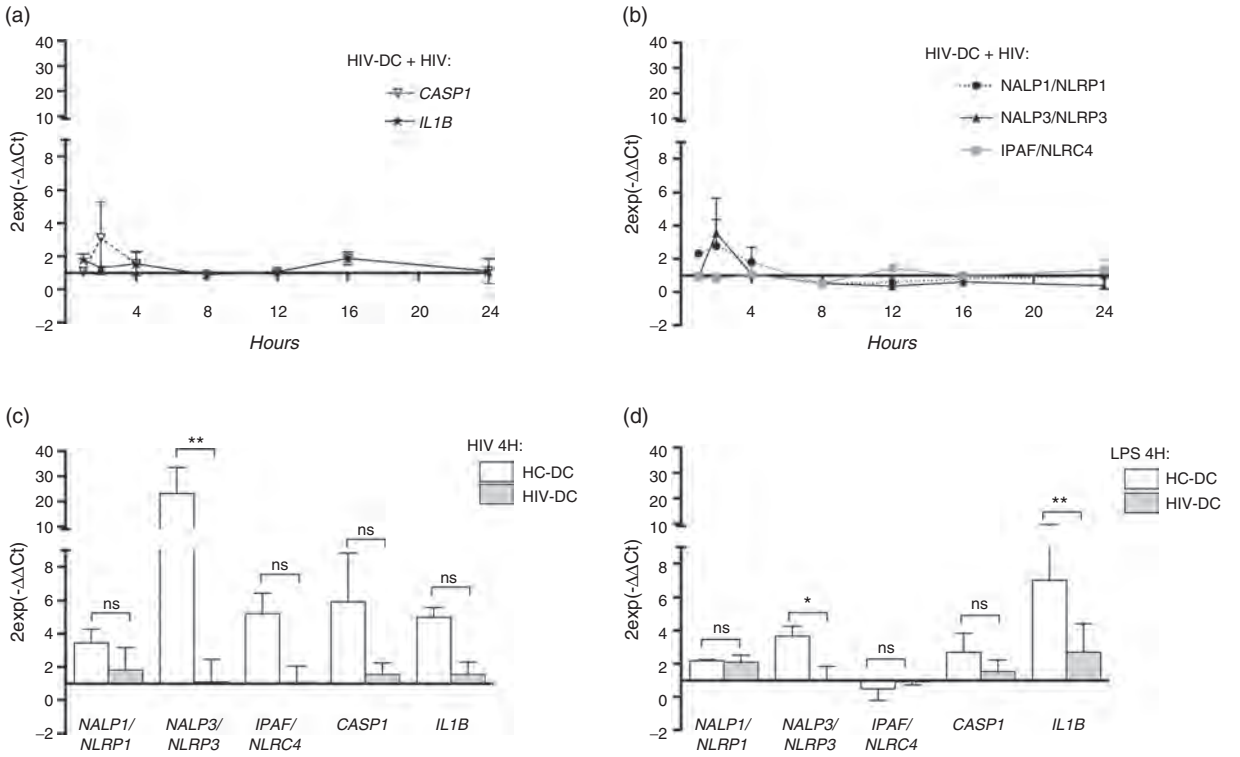

Fig. 3. Inflammasome genes expression in dendritic cells from HIV-positive patients pulsed with alditrithiol-2-inactivated HIV. Time-course of mRNA expression at 1, 2, 4, 8, 12, 16 and 24 h after stimulation of dendritic cells of HIV-positive individuals (HIV-DC) with alditrithiol-2-inactivated HIV (AT-HIV) was reported for *NLRP1, NLRP3* and *NLRC4* (a) and *CASP1* and *IL1B* genes (b). The comparison in genes expression between dendritic cells of healthy individuals (HC-DC) and HIV-DC was reported for 4 h of stimulation with AT-HIV (c) and lipopolysaccharide (LPS) (d). Induced expression of each gene was calculated with respect to the unstimulated condition (*r*) for each individual as $2\exp(-\Delta\Delta Ct)$ in which $\Delta\Delta Ct = \Delta Ct\ HIV_{HIV\text{-}DC}$ or $\Delta Ct\ LPS_{HIV\text{-}DC} - \Delta Ct\ R_{HIV\text{-}DC}$ (resting). All the Ct values were normalized against *ACTB* Ct (ΔCt). Results are expressed as the mean of $2\exp(-\Delta\Delta Ct) \pm SEM$, $n = 20$. One-way ANOVA analysis and Tukey's posttest were used. $^{*}P < 0.05$; $^{**}P < 0.001$; ns = $P > 0.05$. ANOVA, .

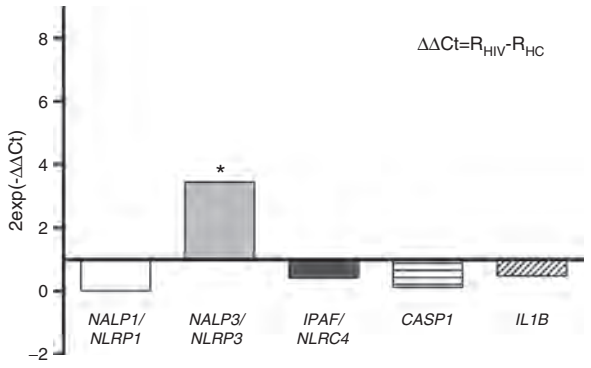

Fig. 4. Basal expression of inflammasome genes in dendritic cells from HIV-positive patients. Difference in basal expression levels of *NLRP1, NLRP3, NLRC4, CASP1* and *IL1B* genes in resting dendritic cells of HIV-positive individuals (HIV-DC) was calculated, comparing the mean expression of each gene in resting HIV-DC and HC-DC as $2\exp(-\Delta\Delta Ct)$ in which $\Delta\Delta Ct = \text{mean } \Delta Ct\ R_{HIV\text{-}DC} - \text{mean } \Delta Ct\ R_{HC\text{-}DC}$. All the Ct values were normalized against *ACTB* Ct (ΔCt) and the average was calculated within the two groups (mean ΔCt, $n = 20$). Results are expressed as $2\exp(-\Delta\Delta Ct)$. One-way ANOVA analysis and Tukey's posttest were used. $^{*}P < 0.05$.

(Tripartite motif-containing protein 5 alpha/TRIM5α, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like/APOBEC-3G, tetherin) [2] and specifically in monocytes and dendritic cells (cyclophilin A, SAM domain and HD domain-containing protein 1/SAMHD1) [12,13] playing a key role in controlling viral replication, our findings focus on the early events of virus–cell interaction and describe an innate anti-HIV mechanism acting between virus entry and nuclear localization.

Surprisingly HIV-DCs were not able to activate inflammasome against AT-HIV or LPS. The HIV-DC unresponsiveness did not correlate with the presence of ART and was not enhanced by the 'maturation' cocktail, suggesting an exhaustive condition related to the chronic viral infection. Despite the fact that treated patients were the majority of the enrolled HIV-positive individuals (70%), and this could be a bias of the study, we did not observe any difference in inflammasome gene expression and IL-1β secretion between naive and treated HIV-positive individuals, reinforcing recent findings about the similarity of phenotypes and response in seropositive individuals regardless of the presence of ART [14].

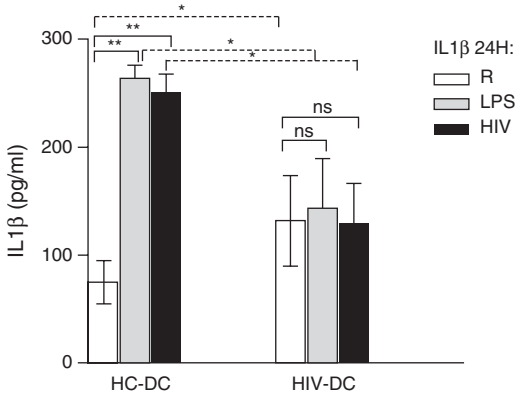

Fig. 5. Interleukin-1β secretion in dendritic cells from healthy individuals and HIV-positive patients pulsed with alditrithiol-2-inactivated HIV. Supernatants of dendritic cells of healthy individuals (HC-DC) and of HIV-positive individuals (HIV-DC) stimulated with alditrithiol-2-inactivated HIV (AT-HIV) or lipopolysaccharide (LPS) for 1, 2, 4, 8, 12, 16 and 24 h were analyzed for the presence of secreted interleukin-1β (IL-1β) as a measure of caspase-1 activity. Secretion of IL-1β (pg/ml) from HC-DC and HIV-DC after 24 h of stimulation with LPS or AT-HIV was reported. Results are expressed as the mean concentration ± SEM. One-way ANOVA analysis and Tukey's posttest were used. $^{*}P < 0.05$; $^{**}P < 0.001$; ns = $P > 0.05$.

Moreover, the cytometric profile of the cells did not allow the identification of such inflammatory 'failure', suggesting the need for new markers of dendritic cell characterization.

Buisson *et al.* [14] have already observed that there was no difference in the dendritic cell phenotype between HIV-infected individuals (naive or in ART) and healthy donors despite the reduced capacity of HIV-DC to stimulate T cells.

The differences between HC-DC and HIV-DC could be explained by considering a possible 'exhaustion' of PBMCs isolated from HIV-positive individuals as already proposed [2,14]. This hypothesis fits well with the high basal *NLRP3* expression observed in resting HIV-DC.

The increased *NLRP3* mRNA expression was previously reported for chronic inflammatory diseases, such as hereditary periodic fevers [15] and Crohn's disease [16], suggesting a common inflammasome response to chronic inflammatory condition, such as HIV-stabilized infection.

The theory of bacterial translocation in HIV-positive individuals [2] could explain the augmented *NLRP3* expression in patients and the missing response to pathogen compounds. Alternatively, similarly to Myxoma virus [5], HIV could directly inhibit inflammasome, leading to a positive feedback mechanism on *NLRP3* expression in resting HIV-DC and to an exhaustion of transcription when dendritic cells were furthermore stimulated.

The identification of NALP3-inflammasome as novel mechanism involved in dendritic cell activation against HIV-1 may open novel questions about the role of inflammasome and inflammation in first steps of HIV infection, as well as in the chronic disease. Recently, we reported the association between the 3′UTR *NLRP3* rs10754558 polymorphism and the risk of HIV infection [6] further sustaining this involvement. The entity of inflammasome activation and IL-1β secretion could drive the full activation of dendritic cell in the first moment of infection (just after the virus–cell contact and its internalization), modulating the antigen presentation and the consequent specific immune response against the virus. Once the infection has been established, the frustrated inflammasome activation in dendritic cell from seropositive individual may interfere with the full maturation of dendritic cell and with an effective immune response. At the same time, the induced hyperexpression of *NALP3* and the moderate but continuous secretion of IL-1β from resting dendritic cells could contribute to a positive feedback mechanism to the chronic inflammation of HIV-patients.

Considering the ongoing research about therapeutic strategies for AIDS involving dendritic cell-based immune-vaccines [1,10], our findings open the question about the possible unresponsiveness of HIV-DC in the in-vitro preparation of the vaccine.

In conclusion, a role for NALP3-inflammasome in the first steps of HIV-1 infection in dendritic cells could be hypothesized, although novel approaches should be considered to fully characterize the HIV/inflammasome interaction. Finally, the unresponsiveness of HIV-DC should be considered in vaccine design, especially those involving dendritic cell manipulation.

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A.P. designed and did the experiments, analyzed the data and wrote the manuscript; L.T.S. cultivated the HIV virus; C.F. did the flow cytometry analysis; T.M.O. and S.C. critically revised the manuscript; A.J.S.D. supervised the study.

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Conflicts of interest

The authors declare no competing financial interests.

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