HIV-1 Induces the First Signal to Activate the NLRP3 Inflammasome in Monocyte-Derived Macrophages

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Key Words

HIV-1 · NLRP3 inflammasome · IL-1 β · Macrophages · Inflammation

Abstract

Background/Aims: Inflammasomes are multimolecular complexes that regulate caspase-1. They act as sensors for endogenous and exogenous signals, and mediate the processing of pro-IL-1 β into its secreted, biologically active form. The NLRP3 inflammasome and IL-1 β are particularly interesting because they are required for efficient control of viral infections. Indeed, HIV-1 induces expression of NLRP3 and IL-1 β in healthy controls, but not in HIV-1-infected patients. Here we evaluate whether HIV-1 can induce activation of the NLRP3 inflammasome. **Methods:** Human primary monocyte-derived macrophages were infected with HIV-1 in the absence or presence of classical NLRP3 inflammasome activators, and IL-1 β release was assessed by ELISA. **Results:** HIV-1 initiates the priming signal for NLRP3 inflammasome activation through the NF- κ B-associated pathway in human primary monocyte-derived macrophages. Furthermore, priming of NLRP3 activation in response to HIV-1 was independent of the viral envelope, since similar results were ob-

served with HIV-1 and pseudotyped HIV-1 lacking the *env* gene. **Conclusion:** Our findings suggest that HIV-1 infection promotes IL-1 β secretion by inducing the first signal for NLRP3 inflammasome activation, a phenomenon that may contribute to AIDS progression.

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Introduction

Alterations in the mechanisms responsible for the control of inflammation have been associated with pathological events. The inflammatory process is highly controlled and IL-1 β plays a central role in this process. The production of IL-1 β requires two signals: (i) transcriptional activation mediated by NF- κ B, which in turn is activated by one of the pattern recognition receptors such as the Toll-like receptors (TLR) [1], and (ii) proteolytic maturation promoted by cytoplasmic complexes called inflammasomes [2]. To date, four inflammasomes have been identified [3], three of which contain proteins of the NOD-like receptor family, including NLRP1, NLRP3 and NLRC4. The fourth inflammasome contains AIM2 that belongs to the pyrin domain and HIN200 domain-con-

taining family [4–7]. All the inflammasome-forming receptor proteins use the adaptor molecule ASC to recruit and efficiently activate caspase-1 in response to several pathogen- or damage-associated molecular patterns [1, 4, 8–13]. Inflammasome activation leads to the cleavage of pro-IL-1 β , to produce the biologically active form, IL-1 β . Other stimuli that activate the NLRP3 inflammasome include ATP, β -amyloid [8], crystals [14, 15], asbestos, silica, aluminum salts [9, 16], residues of necrosis [17] and nanoparticles [18, 19].

The mechanism involved in activating the NLRP3 inflammasome is not well understood. Some studies have proposed a key role of cathepsins (lysosomal proteases) in this process [8, 9, 16]. A second model is based on reactive oxygen species, which could be sensed by the NLRP3 inflammasome [20, 21]. Additionally, it has been reported that alterations in the efflux of potassium [20–23] and calcium mobilization [24, 25] are responsible for NLRP3 inflammasome activation.

Several viruses can activate caspase-1 and induce IL-1 β secretion, highlighting the potential role of the NLRP3 inflammasome in antiviral immune responses [12, 26–28]. However, there are few reports about HIV-1 and inflammasomes, including the association between 3'UTR polymorphism in *NLRP3* and the susceptibility to HIV-1 infection [29]. It was recently reported that HIV-1 induces expression of the mRNA of NLRP3, IL-1 β and caspase-1 in dendritic cells from healthy donors, but not from HIV-1-infected patients [30]. In addition, HIV-1-infected patients show a pro-inflammatory stage with immune hyperactivation, including higher levels of IL-1 β in the serum, which could indeed be explained by the microbial translocation [31–34] (online suppl. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000353902). However, a direct relationship between HIV-1 and NLRP3 inflammasome activation has not been demonstrated. Here, we show that HIV-1 activates the 'priming' signal for NLRP3 inflammasome activation through NF- κ B signaling, and leads to IL-1 β secretion in the presence of specific NLRP3 inflammasome triggers.

Material and Methods

Cell Cultures

Human primary monocyte-derived macrophages (MDM) obtained from healthy donors were used here. Human macrophages were used because they are a cellular target for HIV-1 infection in which NLRP3 inflammasome activation has been previously studied. CD14⁺ monocytes were acquired by elutriation from PBMCs [35]. Differentiation into macrophages was obtained after 7 days

of culture at 37° and 5% CO₂ in the presence of 10 ng/ml GM-CSF (PeproTech). Monocyte differentiation into MDM was confirmed by flow cytometry on an LSR II flow cytometer (BD Biosciences) and analyzed using FlowJo software (Tree Star, Inc.), using macrophage markers (CD68/CD14) (data not shown). In all the reagents used no endotoxin could be detected, using the Limulus amoebocyte lysate (LAL) assay (data not shown).

Viruses

293T cells were transfected with 100 ng of pNL4-3 or pNL4-3 Δ env in the presence of the pVSV-G envelope vector (vesicular stomatitis virus glycoprotein G), using GeneJuice (EMD Biosciences, San Diego, Calif., USA). After 48 h, the supernatant was harvested and filtered through 0.22- μ m filters. This strategy yielded two types of viruses: HIV-1_{VSV-G} and the pseudotyped HIV-1 Δ env_{VSV-G}. This means that HIV-1 with or without envelope glycoproteins and both with VSV-G to increase the efficiency of infection, reaching 60% in all the experiments, compared to 2–4% without VSV-G protein, as previously reported [36–38]. Viral titers were quantified by a radioactive reverse transcriptase assay, to determine the amount of infectious particles [39]. Viral stocks were treated with DNase-I to remove residual DNA, as previously described [40].

In vitro HIV-1 Infections and NLRP3 Inflammasome Stimulation

Differentiated MDM were stimulated to evaluate both signals of NLRP3 inflammasome stimulation as previously reported [1]. In brief, priming was initiated with 50 pg/ml LPS or with a pulse of HIV-1_{VSV-G} or HIV-1 Δ env_{VSV-G} (300,000 reverse transcriptase units (RTU)). Triggers of the second signal were added 2 h later: 5 mM ATP, 10 μ M nigericin, 50 μ g/ml silica, 100 mg/ml alum, 20 μ g/ml monosodium urate crystals (MSU), and 1 μ M R848 or 100 ng/ml LPS, for 4 h (1 h for ATP and nigericin) [41]. In addition, HIV-1 was also used to stimulate the second signal.

ELISA

IL-1 β and IL-6 secretion was quantified using ELISA (BD Biosciences) and expressed as pg/ml. IL-1 β detection was performed in the supernatants to quantify only the processed form of the cytokine.

Results

HIV-1 Induces the Production of IL-1 β in Response to NLRP3 Inflammasome Agonists

MDMs primed with LPS, HIV-1_{VSV-G} or HIV-1 Δ env_{VSV-G} and then treated with NLRP3 inflammasome activators secreted high levels of IL-1 β (fig. 1). HIV-1 Δ env_{VSV-G} was used to compare the effects of HIV-1 priming in the absence of the envelope glycoproteins. Interestingly, a similar IL-1 β production in response to LPS or HIV-1 and the classical NLRP3 inflammasome activators was detected. No IL-1 β production was observed in MDM stimulated only with the priming signal or with inducers of the second signal in the absence of priming.

Fig. 1. HIV-1 induces the production of IL-1 β in response to NLRP3 inflammasome activators. MDMs were primed with LPS (50 pg/ml) or HIV-1 (HIV-1_{VSV-G} or HIV-1 Δ env_{VSV-G}) (300,000 RTU) for 2 h. Subsequently, the cells were treated with activators of the second signal for NLRP3 inflammasome: ATP (5 mM), nigericin (10 μ M), silica (50 μ g/ml), alum (100 mg/ml) and MSU (20 μ g/ml). Four hours later the production of IL-1 β was quantified in the supernatants by ELISA. Representative results of three independent experiments are shown as mean and standard deviation. Comparisons were performed using the Kruskal-Wallis ANOVA tests and Dunn's post-tests. The level of significance was * $p < 0.05$.

Furthermore, no differences were detected in the secretion of IL-1 β based on the viral type used, HIV-1_{VSV-G} or HIV-1 Δ env_{VSV-G} (fig. 1). Similar results were observed in response to silica, alum or MSU, but in these cases, IL-1 β production was greater when LPS was used as priming signal compared to HIV-1 primed cells (fig. 1). Moreover, in the absence of a second signal activator, HIV-1 was unable to induce IL-1 β release (fig. 1). These results suggest that HIV-1 can induce at least one of the two signals for NLRP3 inflammasome activation in vitro.

HIV-1 Fails to Induce the Second Signal for NLRP3 Inflammasome Activation

To determine whether HIV-1 stimulates the second signal involved in NLRP3 inflammasome activation, MDMs were primed with LPS (50 pg/ml) or HIV-1_{VSV-G} or HIV-1 Δ env_{VSV-G} for 2 h. The cells were then treated with activators of the second signal for the NLRP3 inflammasome [42], as well as HIV-1_{VSV-G}. Our results show that when HIV-1 was used as priming and second signal, the MDMs were unable to secrete IL-1 β , suggesting failure to activate the NLRP3 inflammasome. In contrast, classical inflammasome activators such as ATP and silica, and alternative activators such as LPS (100 ng/ml) and R848, induced higher IL-1 β production than HIV-1 (fig. 2a). These results suggest that early phases of HIV-1 infection are unable to induce the second signal for NLRP3 inflammasome activation.

HIV-1 Induces the Activation of NF- κ B, as Determined by IL-6 Production

The induction of the first signal for NLRP3 inflammasome activation requires the activation of NF- κ B, whose

signaling triggers the expression of molecules including NLRP3, pro-IL-1 β and IL-6 [1]. Therefore, the ability of HIV-1 to stimulate NF- κ B signaling was determined by quantifying IL-6 production by MDM. As shown in figure 2b, the stimulation of MDM with HIV-1 as the first signal (fig. 2b) induced the production of IL-6 to a similar level as LPS. As one would expect, ATP and MSU do not have a notorious effect on IL-6 release, but TLR stimulation induces a higher production of IL-6, even in the absence of the priming signal. These results indicate that HIV-1 can activate the production of IL-6 through NF- κ B signaling, which is in agreement with the results presented in figure 2a. Thus, HIV-1 activates the first signal for NLRP3 inflammasome through the NF- κ B signaling pathway.

Discussion

The NLRP3 inflammasome is one of the components involved in the inflammatory process and constitutes a new member of the innate immune system receptors involved in virus recognition, although its relationship with HIV-1 is only beginning to be studied. Indeed, Pontillo et al. [30] have described that HIV-1 induces the expression of NLRP3 inflammasome components and IL-1 β secretion in dendritic cells from healthy individuals but not from HIV-1-infected patients, suggesting a role of the inflammatory process in disease progression. Furthermore, it has been reported that the plasma of HIV-1-infected patients presents increased IL-1 β levels during all the stages of infection ([33] and online suppl. S1). However, the mechanism involved in

Fig. 2. HIV-1 induces the production of IL-6 in MDM, but does not activate the second signal for NLRP3 inflammasome activation. MDMs were primed with LPS (50 pg/ml) or HIV-1 (HIV-1_{VSV-G} or HIV-1Δenv_{VSV-G}) for 2 h. Subsequently, the cells were treated with classical activators of the second signal for NLRP3 inflammasome: ATP 5 mM, MSU 20 μg/ml, silica 50 μg/ml, with LPS 100 ng/ml (TLR4 ligand), R848 1 μM (TLR7 ligand), or with HIV-1_{VSV-G} 300,000 RTU. Four hours later the production of IL-1β (a) and IL-6 (b) was quantified in the supernatants by ELISA. Representative results of three independent experiments are shown as mean and standard deviation. Comparisons were performed using Kruskal-Wallis ANOVA tests and Dunn's post-tests. The level of significance was * $p < 0.05$.

the recognition of HIV-1 by the NLRP3 inflammasome is not well understood. Here we show that HIV-1 induces the first signal required for NLRP3 inflammasome activation, since high levels of IL-1β secretion were detected in the supernatants of MDM stimulated with HIV-1 as priming signal, and the classical agonists of NLRP3 inflammasome as second signal (fig. 1). These results indicate that HIV-1 induces the assembly of the active NLRP3 inflammasome that leads to IL-1β production in the presence of specific NLRP3 inflammasome triggers. Indeed, during early moments of HIV-1-cell interactions, a specific expression of the NLRP3 inflammasome components occurs in dendritic cells [33]. This mechanism could help HIV-1 for acute replication and spread. During the chronic phase, other mechanisms are also implicated in the HIV-1 replication and progression, as immune hyperactivation, microbial translocation, or immunosuppression, among other things. However, when HIV-1 was used as priming and second signal, the MDMs were unable to produce IL-1β (fig. 2a). Together the results suggest that HIV-1 acts as priming signal to activate the NLRP3 inflammasome, but is not sufficient to induce IL-1β production on its own. HIV-1 infection is a multifactorial

event that may be modulated by several components in the host, environment and the virus itself.

It has been reported that the NLRP3 inflammasome can be activated in response to both RNA and DNA [4, 43]. Since the HIV-1 genome consists of two RNA molecules that in turn are reverse transcribed into a cDNA molecule, these replication intermediates are real candidates for the activation of the NLRP3 inflammasome. The experiments presented here do not allow us to assess whether viral replication could affect activation of the NLRP3 inflammasome. However, they indicate that exposure to HIV-1 was sufficient to activate NF-κB-mediated intracellular signaling and subsequent IL-6 production, consistent with previous studies showing cellular activation and inflammatory response induced by viral components, such as gp120 and the genomic RNA [44, 45]. Our results also indicate that activation of the NLRP3 inflammasome is independent of the HIV-1 envelope protein, since similar results were observed in MDM stimulated with HIV-1_{VSV-G} or HIV-1Δenv_{VSV-G}. The NLRP3 inflammasome can be activated by viruses or their products [46], including varicella-zoster virus [47], influenza virus [12, 27], hepatitis C virus [48] and adenovirus type 5 [49, 50]. In addition to viral models, other

infectious agents have been associated with NLRP3 inflammasome activation, including immunopathogenesis mechanisms, such as *Aeromonas* spp. [51] and *Borrelia* spp. [52] that induce the expression of IL-1 β and IL-18, altering the development of inflammatory immune response. Several microorganisms could be present during the course of HIV-1 due to an immunosuppression state, as well as PAMPs, associated to microbial products translocation from gut, which is a key feature in HIV-1 pathogenesis. However, some microorganisms can also block the activation of the NLRP3 inflammasome to favor their persistence in the host. For instance, the viral protein Orf63 of Kaposi's sarcoma-associated herpesvirus acts as a viral homologue of NLRP1, blocking the function of the NLRP1 and NLRP3 inflammasomes, decreasing the inflammatory response, and promoting viral reactivation [53]. In terms of endogenous ligands, the cholesterol crystals could be present in HIV-1-infected patients, taking into account the increased risk of cardiovascular diseases among these patients, due to high cholesterol levels in serum [54–56].

Little is known regarding the relationship between HIV-1 and inflammasomes. There is increased expression of IL-1 β in HIV-1-infected individuals [31, 32, 34], but information about the mechanisms that promote IL-1 β production is sparse. It has also been reported that susceptibility to HIV-1 infection could be associated with polymorphisms in the 3'UTR of *NLRP3* [29]; this is the first report that correlates the NLRP3 inflammasome and HIV-1 in macrophages, although no functional studies have been performed. A better understanding of this pathway will advance the development of new strategies against HIV-1 infection. Our results suggest that HIV-1 is able to act as a first signal inducer for NLRP3 inflammasome activation, which could serve in the development of new therapeutic agents to enhance current antiviral strategies. However, our results also show that HIV-1 could not induce the second signal for NLRP3

inflammasome activation, in contrast to the results observed with classical (ATP/silica) and non-classical (LPS/R848) activators that induce IL-1 β production in primed MDMs. Consistent with previous reports [57], this study shows that HIV-1 activates NF- κ B intracellular signaling as determined by IL-6 production. They support the hypothesis concerning the role of HIV-1 in the induction of the first signal, since NF- κ B is responsible for activating the transcription of the genes encoding NLRP3 inflammasome components, such as pro-IL-1 β and NLRP3.

Conclusion

These results extend our understanding of the mechanisms of evasion or even exploitation of the immune response to favor viral persistence and pathogenesis. While the work reported here establishes the early phase of HIV-1 infection, as an important priming signal, additional studies are needed for a comprehensive understanding of the NLRP3 inflammasome in the pathogenesis of HIV-1.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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