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Porcine Endogenous Retrovirus Induces CXCL10 in Human Monocytes and Monocyte-Derived Primary Cells

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Keywords
Porcine endogenous retrovirus · CXCL10 · Innate immunity · Xenotransplantation · Monocytes

Abstract
Introduction: Pigs are suitable donor species for xenotransplantation and biological materials from these animals are used for this purpose for many years. A major risk of xenotransplantation is a zoonosis by transspecies transmission of animal viruses. In this regard, porcine endogenous retroviruses (PERVs) are of paramount importance because some of them are able to infect human cells and could induce innate immune responses. ***Methods:*** Using a replication-competent polytropic PERV-A/C strain, we have analysed the induction of innate immune responses by this virus in human monocytes, monocyte-derived macrophages, and monocyte-derived dendritic cells. ***Results:*** PERV-A/C elevates the expression of the C-X-C motif chemokine 10 (CXCL10) up to 1,000-fold in human monocytes and monocyte-derived primary cells. In comparison to CXCL10, the levels of interferon-β (IFN-β) and interferon-stimulated gene 54 (ISG54) were almost unchanged. Heat-inactivated virus did not induce CXCL10 expression. Neither treatment with the reverse transcriptase inhibitors azidothymidine (AZT) and stavudine (d4T) nor treatment with the integrase inhibitor raltegravir (RAL) reduced the activation levels. Furthermore, depletion of SAM domain and HD domain-containing protein 1 (SAMHD1), a restriction factor that blocks PERV-A/C infection at the level of reverse transcription in these myeloid cells, had no significant effect on the CXCL10 induction level. These results imply that innate immune sensing leading to the strong CXCL10 response occurs at an early step of the replication process and does not require products of reverse transcription. Inhibition of Janus kinases (JAKs) by AT9283 prevented the observed CXCL10 induction by the virus, providing evidence that the JAK-STAT signalling pathway is involved in the CXCL10 response in theses myeloid cells. ***Conclusion:*** Our findings highlight PERVs as inducers of the pro-inflammatory chemokine CXCL10 and other innate immune responses in human monocytes and derived cells with potential implications in the context of xenotransplantation.

Introduction

The number of individuals waiting for an allotransplant is increasing and many people die before they receive the organ they need. Xenotransplantation is a potential way to relieve or overcome this shortage. Pigs are

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considered to be a suitable transplant donor for many reasons. They are physiologically similar to humans, are easily cloned, genetically modified, and have a relatively short reproduction time [1, 2]. On the other hand, pigs can harbour infectious agents that endanger the human transplant recipient especially if immunosuppressed for prevention of organ rejection. Along these lines a zoonosis by transspecies transmission of porcine endogenous retroviruses (PERVs) to the human recipient and PERV-induced immunosuppressive effects are considered as potential risks [3]. PERVs are integrated within the genome of all pigs, but the number of proviral loci and their expression levels vary by breed [4–6]. There are three basic subtypes of PERVs known. PERV-A and PERV-B are polytropic and replicate in porcine cells and cells of other species including human cells. In contrast, PERV-C is ecotropic and infects only porcine cells [7]. An additional form with high infectivity and polytropism is known. These are recombinants of PERV-A and C (PERV-A/C), which were discovered in different organs and tissues of pigs but not in the germline [8, 9]. The principal ability of PERVs to productively infect a variety of human cell types, including the human embryonic kidney-293 (HEK-293) cell line and normal human dermal fibroblasts (NHDFs), has been described previously [10, 11]. Nevertheless, despite numerous xenotransplantations with porcine organs, tissues and other biological materials such as pancreatic islets cells [12, 13], nerve cells [14] or porcine skin grafts [15], no infections with any of the PERV variants were documented. A potential reason is innate immune responses by the transplant recipients that are considered the first line of defence against invading pathogens.

Innate immune responses are induced by pattern recognition receptors (PRRs) that are activated by pathogen-associated molecular patterns (PAMPs) [16]. PAMPs are molecular motives in proteins, nucleic acids, and other macromolecules of agents. PRRs are present in different cellular compartments, mostly in endosomes and the cytoplasm [17–19]. These sensors signal through specific adaptor molecules to activate critical transcription factors, such as nuclear factor-κB (NF-κB) and interferon regulatory factor 3 (IRF 3) which eventually results in the production of interferons (IFNs) and other pro-inflammatory cytokines [20]. IFNs themselves induce a plethora of cytokines including the interferon-γ-induced protein 10 (IP-10) also referred to as CXCL10, as it belongs to the CXC chemokine family [21]. Upon stimulation by IFN-γ, IFN-α, IFN-β, interleukin-2, and other cytokines CXCL10 is primarily secreted by monocytes, other myeloid cells, T-lymphocytes, natural killer cells, and endothelial cells, among others [22, 23]. CXCL10 binds to the CXCR3 receptor to exert its biological effects associated with inflammation, infectious diseases and tumour development [24]. CXCL10 reaches very high plasma levels in HIV infected individuals and the levels stay elevated even in patients under successful antiretroviral therapy. It is considered an important pro-inflammatory factor in the disease. The chemokine facilitates herpes simplex virus type 2 (HSV-2) infections [25] and the high expression of CXCL10 in COVID-19 is implicated in the cytokines storm, which often leads to organ failures [26].

In this study, we investigated the immune response to PERV in human monocytes, monocyte-derived dendritic cells (MDDC) and macrophages (MDM). We focus on the induction of IFN-β, CXCL10, and interferon-stimulated gene 54 (ISG54) as characteristic genes early involved in the activation of the innate immune system.

Materials and Methods

Cells and Cell Culture

HEK-293T cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. HEK-293T cells were grown in cell culture flasks at 37°C, 5% CO_2, and 98% humidity. Human peripheral blood mononuclear cells (PBMC) were purified from healthy blood donors (German Red Cross, Berlin) by ficoll (Sigma) density gradient centrifugation. PBMCs appeared as a thin white layer above ficoll and below serum, which were removed by pipetting and placed in a new 50 mL conical tube and washed two times with PBS. PBMCs were routinely counted using a Neubauer counting chamber and seeded in different cell culture flasks and plates depending on the type of cells to be obtained by differentiation. To obtain monocytes, PBMCs were seeded in 12-well plates for 90 min to allow adherence. Monocytes were cultured in DC-medium (RPMI-1640 supplemented with 5% pooled human serum, 1 mM HEPES, and 1% penicillin/streptomycin). MDM and MDDC were differentiated from monocytes as previously described [27]. Briefly, MDM were differentiated from monocytes by using 50 ng mL^{-1} GM-CSF (Peprotech) for 6 days in 12-well plates. DC-medium was changed each 2 days and 50 ng mL^{-1} GM-CSF were added at the same time. To differentiated monocytes to MDDC, 50 ng mL^{-1} GM-CSF and 20 ng mL^{-1} interleukin-4 (Peprotech) were added to the cells each 2 days for 6 days. THP-1-Dual™ cells (InvivoGen) were derived from the human THP-1 monocyte cell line by stable integration of two inducible reporter constructs. These reporters allow the simultaneous study of the NF-κB pathway by monitoring the activity of secreted alkaline phosphatase (SEAP) and the IRF pathway by measuring the activity of a secreted luciferase (Lucia). THP-1-dual reporter cells were grown in RPMI-1640 culture medium. The innate immune activators R848 (InvivoGen), Poly I:C (InvivoGen), and IFN-β (R&D systems) were used in all experiments in a final concentration of 10 μM. The signalling pathway inhibitors parthenolide (Sigma), AT9283 (Selleckchem), BX-795

(Sigma) were applied at a concentration of 1 μM. THP-1 cells were treated with these drugs for 24 h before analysing SEAP and Lucia production.

Preparation of Viral Stocks

In order to infect human primary cells, viral stocks were prepared. For this purpose, HEK-293T cells were seeded in T25 cell culture flasks. When cell confluency reached 25–50%, PERV-A/C was added to the cells. The endogenous PERV-A/C was initially isolated from porcine PBMC and has been described earlier [28]. Cells were incubated for 3 days with the viruses. Afterwards, they were trypsinized and further cultured in larger flasks for 3 days. Following the 10th day post infection, cell culture supernatants were harvested five times every 3rd day. Supernatants were ultracentrifuged, resulting pellets resuspended, filtered (0.2 μm), and aliquots stored at −80°C. The amount of virus was determined by titration using a real-time PCR approach, as described earlier [27].

Generation of Virus-Like Particles

For virus-like particle (VLP) production HEK-293T cells were seeded in 10 cm cell dishes and transfected with the following expression plasmid: pMDLgag/pol (10 μg), pRSVrev (1 μg), VSVg (2 μg), and pcDNA or pVPXmycHis (2 μg) [27]. Plasmids were diluted in 1 mL of serum-free DMEM and the equal amount (15 μg) of polyethylenimine (PEI) (Polyscience) was added to the solution and incubated at room temperature for 15 min. The solution was then added dropwise to the cell culture dishes. After 6 h of incubation the medium was exchanged. 48 h after transfection the viruses were harvested concentrated by ultracentrifugation, aliquoted, and quantified using a real-time PCR approach.

Infection Experiments

Human primary cells were spin-infected with $10^{5.7}$ TCID 50 mL^{-1} of PERV-A/C treated for 10 min with benzonase (Sigma) to eliminate contaminating plasmid DNA. 48 h later, medium was removed and cells were washed two times with PBS before lysis for subsequent analysis. In experiments with VLPs, cells were infected in a similar way with these particles 1 h before PERV-A/C was added. Treatment with 10 μM reverse transcriptase inhibitors (azidothymidine [AZT], stavudine [d4T]) or integrase inhibitor (raltegravir [RAL]) (Sigma) dissolved in DMSO was done 1 h before and during PERV-A/C infection for 1 h. Likewise, MDM and MDDC were treated with 1 μM of parthenolide, AT9283, or BX-795 before and during PERV-A/C infection.

Reverse Transcription Quantitative Real-Time PCR

RNA isolation from cells was performed using the RNeasy mini-kit (Qiagen) as described in the manual of the manufacturer. RNA was determined using a NanoDrop1000 spectrometer (Thermo Scientific). Reverse transcription of isolated cellular RNA was done with a RevertAid H minus first strand cDNA synthesis kit (Thermo Scientific). Subsequently, relative levels of the cytokine cDNAs were determined applying the ΔΔCT method and normalizing the CT values to the CT values of the housekeeping gene GAPDH, as previously described [27]. Duplex real-time PCRs were run using the following gene expression assays supplied by Thermo Scientific: CXCL10 (Assay ID Hs00171042_m1), IFN-β (Assay ID Hs01077958_s1), ISG54 (Assay ID Hs00533665_m1, and GAPDH (Asssay ID Hs02786624_gl). The cycling conditions were 95°C for 15 min, followed by 39 cycles of 95°C for 10 s and 60°C for 20 s. The sensiFAST probe No-ROX qPCR Kit (Bioline) was used. All PCR reactions were carried out using the BioRad CFX96 system.

Western Blot

Cells were washed twice with cold PBS 48 h post infection and either lysed directly in the well or pelleted and resuspended in lysis buffer (100 mM Tris, 30 mM NaCl, 0.5% Nonidet P-40). After incubation on ice for 10 min, protein loading buffer was added to lysates. Samples were boiled at 95°C for 5 min and run on a 12% denaturing SDS polyacrylamide gel. Proteins were transferred onto a nitrocellulose membrane (BioRad), blocked with blocking solution (Rockland), and incubated with primary antibodies. As primary antibodies mouse anti-SAMHD1 (OriGene) diluted 1:1,000 in blocking solution and rabbit anti-GAPDH (Sigma), diluted 1:5,000 in blocking solution were used. As secondary antibodies IRDye 800 goat anti-mouse or IRDye 680 goat anti-rabbit (Li-Cor), diluted 1:10,000 in blocking solution were applied. Blots were scanned using an Odyssey infrared scanner (Li-Cor).

Results

PERV-A/C Induces a Strong CXCL10 Response in Human Monocytes and Monocyte-Derived Cells

To assess the general ability of PERVs to induce innate immune responses in primary human myeloid cells, we have used a replication-competent PERV-A/C virus for experiments. We started the analysis with monocytes from three healthy donors that were isolated from PBMCs. As representative cytokines that are typically induced during innate immune activation by viruses we have chosen CXCL10, IFN-β, and ISG54. The relative differences in the mRNA levels of these cytokines were quantified by real-time PCR 48 h post infection. The results show that PERV-A/C increased the CXCL10 mRNA level compared with non-infected cells in all three donors about 100-fold (Fig. 1). We have recently shown that heat treatment of PERV-A/C (10 min at 80°C) prevents the generation of reverse transcription (RT) products in human monocytes, MDM, and MDDC even if SAMHD1 is degraded [27]. Here we report that heat treatment of the virus before infection prohibited almost completely the CXCL10 upsurge. This indicates that the CXCL10 induction is not caused by heat stable impurities (e.g., lipopolysaccarides [LPS]) in the virus preparation (Fig. 1a).

We have previously also demonstrated that SAMHD1 is able to prevent PERV-A/C infection of human myeloid cells and the presence of PERV cDNA in infected cells requires its degradation [27]. Therefore, we set out to analyse the role of this restriction factor in the CXCL10 induction in monocytes. For this, we have degraded SAMHD1 with SIVmac239 Vpx that was brought into the

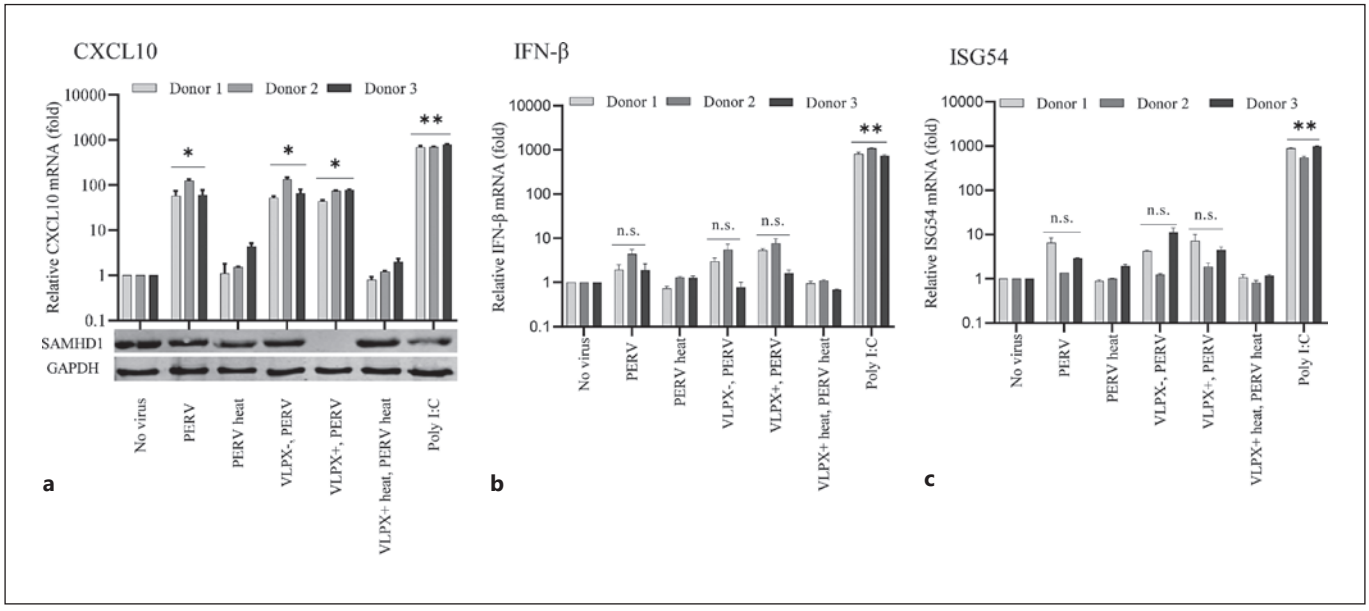

Fig. 1. Induction of innate immune responses in human monocytes by PERV-A/C. Monocytes from three healthy donors were treated with PERV-A/C and levels of (**a**) CXCL10 mRNA, (**b**) IFN-β mRNA, and (**c**) ISG54 mRNA were analysed 48 h later. Reverse transcribed cytokine mRNA was quantified relative to GAPDH. The mRNA in untreated cells was set to one and used to calculate the fold difference. Heat-inactivated PERV-A/C served as negative control and poly I:C transfection was used as a positive control for innate immune activation. $VLPX^+$ indicates pre-treatment with VLPs that carry Vpx and VLP^- denotes pre-treatment with VLPs lacking Vpx. SAMHD1 degradation by Vpx in cells from donor one is documented by Western blot. Mean values of triplicates and SD of a representative experiment are shown. *$p <$ 0.05; **$p < 0.005$; n.s., not significant; unpaired *t* test.

cell by VSV-G pseudotyped HIV-derived VLPs ($VLPX^+$) 1 h before PERV-A/C was added. The same VLPs without Vpx cargo ($VLPX^-$) were used as control. Although Vpx reduced SAMHD1 to levels undetectable by Western blot, this had no significant effect on the increase in the CXCL10 expression. As expected, PERV-A/C and heat inactivation of both $VLPX^+$ and PERV-A/C did also not induce CXCL10 (Fig. 1a). In agreement with our previous findings [27], heat inactivation of PERV-A/C in samples pre-treated with VLPX ($VLPX^+$) decreased the presence of PERV-A/C cDNA in monocytes of the three donors over 100-fold down to background levels (determined with "no virus" samples, data not shown).

In contrast to CXCL10, the IFN-β response to PERV-A/C in monocytes was only slightly increased in two of the donors but did not reach a significant level in contrast to the stimulation by Poly I:C used as control. No effect on the IFN-β expression has been seen by addition of VLPs and subsequent downregulation of SAMHD1 (Fig. 1b). Results similar to the IFN-β response were also obtained by measuring the ISG54 expression to PERV-A/C in monocytes (Fig. 1c).

Next, we sought to analyse the CXCL10, IFN-β, and ISG54 responses of MDM to PERV-A/C. As shown in Figure 2a, CXCL10 mRNA levels were even somewhat higher in MDM of the three donors compared with monocytes. In one of the donors (donor 3), CXCL10 induction reached almost a level of 1,000-fold (Fig. 2a). Knockdown of SAMHD1 by the accessory protein Vpx did not change the CXCL10 induction and treatment with heat-inactivated PERV-A/C had no significant effect on the CXCL10 mRNA level (Fig. 2a). Consistent with the results seen with monocytes, neither IFN-β (Fig. 2b) nor ISG54 (Fig. 2c) were substantially induced in human MDM by this pig retrovirus.

As a third primary cell of the myeloid lineage, we investigated the induction of the three cytokines by PERV-A/C in MDDC. As with monocytes and MDM, PERV-A/C increased the CXCL10 mRNA 2 days post virus addition over 100-fold in MDDC as well (Fig. 3a). As with monocytes and MDM, pre-treatment of MDDC with $VLPX^+$ resulted in a significant presence of PERV-A/C cDNA in infected cells that was not observed if heat-inactivated PERV-A/C was used (data not shown). Downregulation of SAMHD1 had also no additional effect in

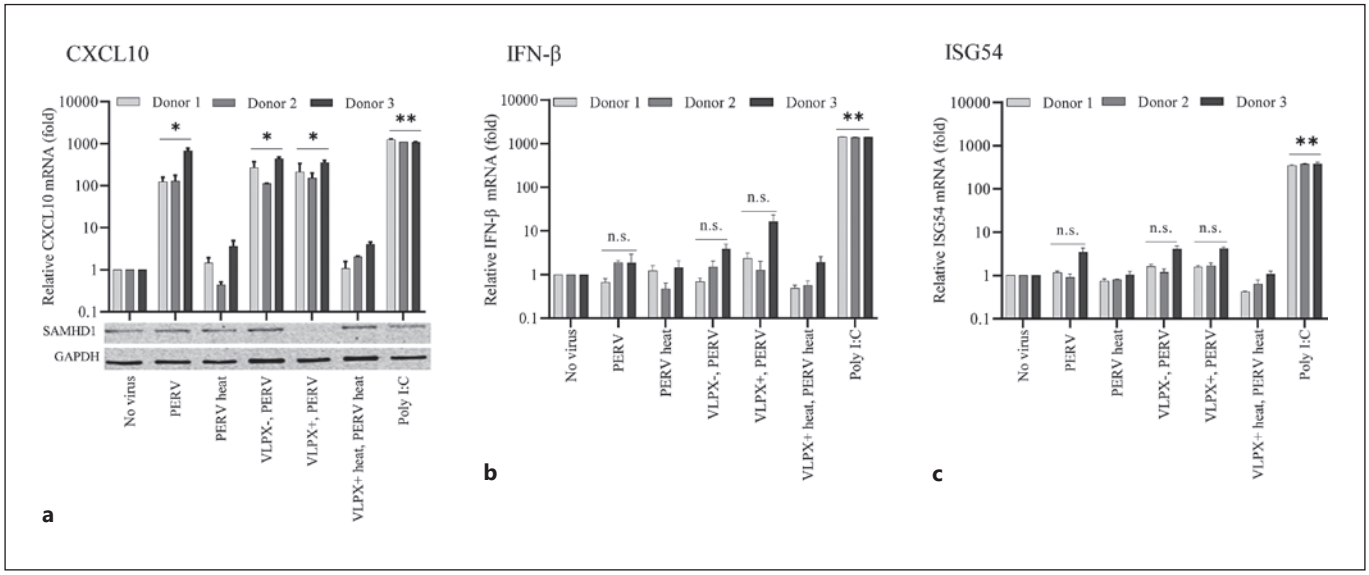

Fig. 2. CXCL10 is significantly induced in MDM by PERV-A/C. Monocytes from three healthy human donors were isolated and differentiated into MDM for 6 days by adding GM-CSF. The experiments were performed as described in Figure 1 with monocytes. **a** Induction of CXCL10 mRNA. **b** Induction of IFN-β mRNA. **c** Induction of ISG54 mRNA. Mean values of triplicates and SD of a representative experiment are shown. *$p < 0.05$; **$p < 0.005$; n.s., not significant; unpaired *t* test.

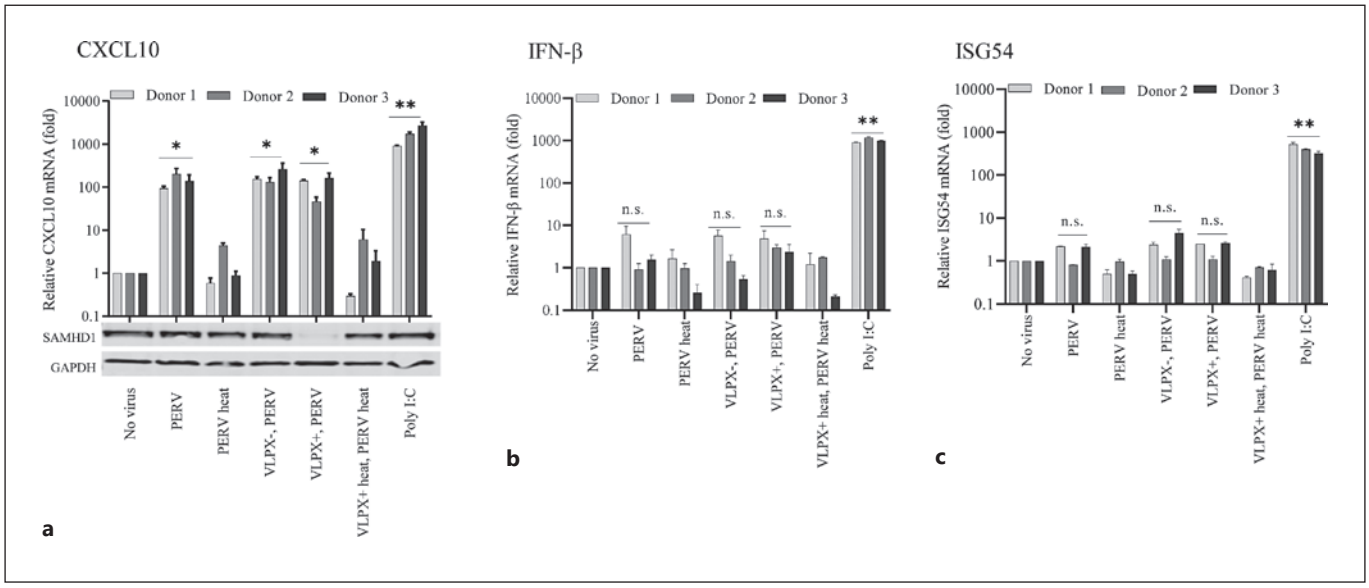

Fig. 3. PERV-A/C induces CXCL10 transcription in MDDC. MDDC derived from monocytes of three healthy donors by addition of GM-CSF and interleukin-4 were used. The experiments with MDDC were performed as described in Figure 1 with monocytes. **a** Induction of CXCL10 mRNA. **b** Induction of IFN-β mRNA. **c** Induction of ISG54 mRNA. Mean values of triplicates and SD of a representative experiment are shown. *$p < 0.05$; **$p < 0.005$; n.s., not significant; unpaired *t* test.

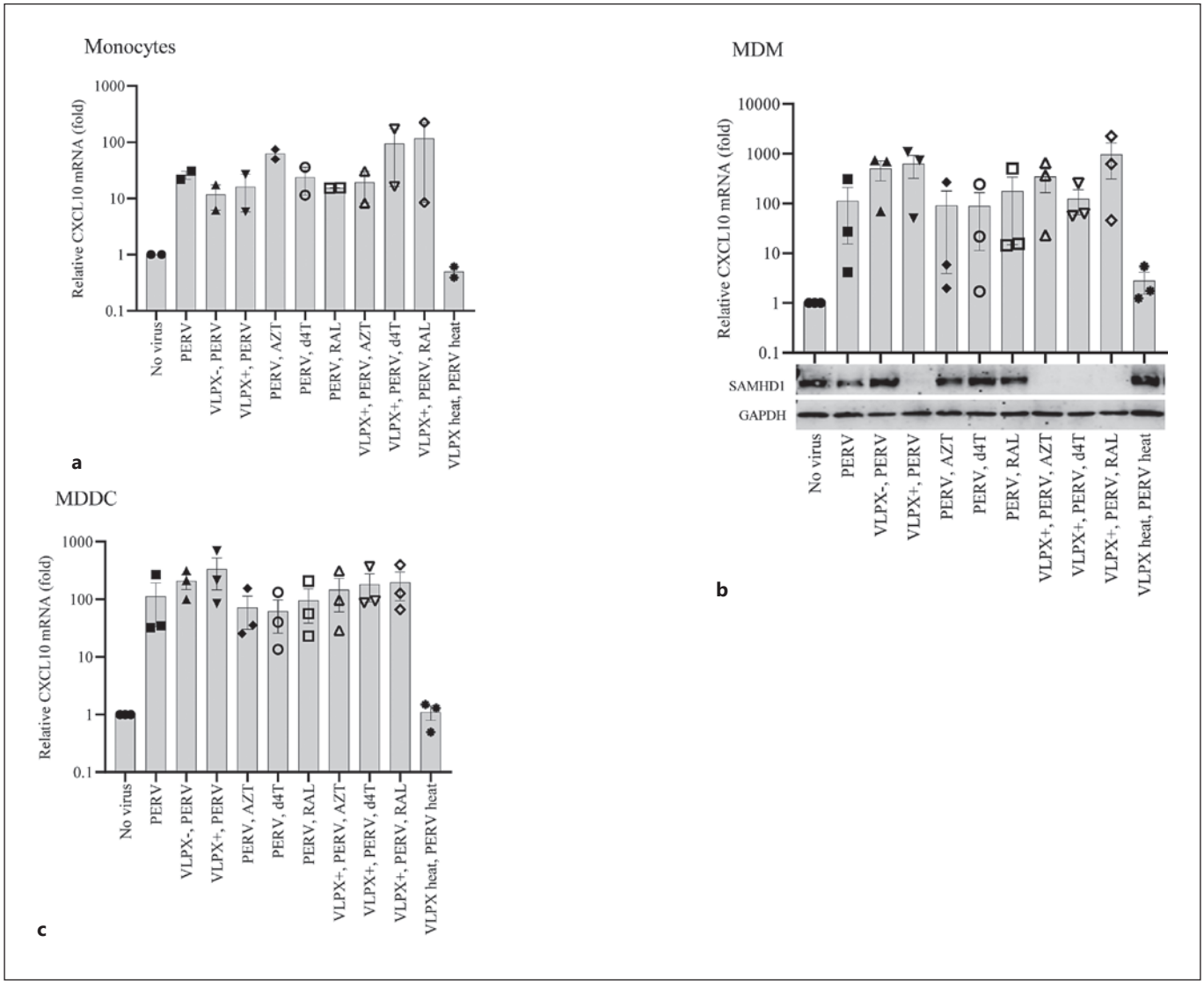

Fig. 4. CXCL10 stimulation by PERV-A/C in human myeloid cells occurs at a replication step prior to reverse transcription. For inhibition of reverse transcription AZT has been used. The two antiretroviral drugs d4T and RAL serve as controls. Levels of CXCL10 mRNA were analysed in (**a**) monocytes, (**b**) MDM, and (**c**) MDDC from three donors 48 h after addition of PERV-A/C. Reverse transcribed CXCL10 mRNA was quantified relative to GAPDH. The mRNA in non-infected cells was set to one and used to calculate the fold difference using the ΔΔCT method. Heat-inactivated PERV-A/C served as negative control. VLPX$^+$ indicates pre-treatment with VLPs that carry Vpx and VLP$^-$ denotes pre-treatment with VLPs lacking Vpx. SAMHD1 degradation by Vpx in MDM from donor one is exemplified by Western blot. Mean values from triplicates +/− SD of a representative experiment are shown.

MDDC on CXCL10 expression and IFN-β and ISG54 were only very marginally induced in some of the donors (Fig. 3b, c).

PERV-A/C Sensing Leading to CXCL10 Stimulation Does Not Require Reverse Transcription

In order to shed light on the replication step at which PERV-A/C induces the CXCL10 response in monocytes and monocyte-derived primary cells, we have pre-treated these cells with 10 μM of AZT, d4T, and RAL 1 h before and during infection with PERV-A/C. It is known that AZT inhibits PERV with an IC50 in the range of 0.015–0.25 μM in cell-based assays [29–31]. We previously also found that 10 μM AZT strongly inhibit PERV-A/C reverse transcription in these myeloid cells [27]. As controls, we have used a second RT-inhibitor (d4T) that in contrast to

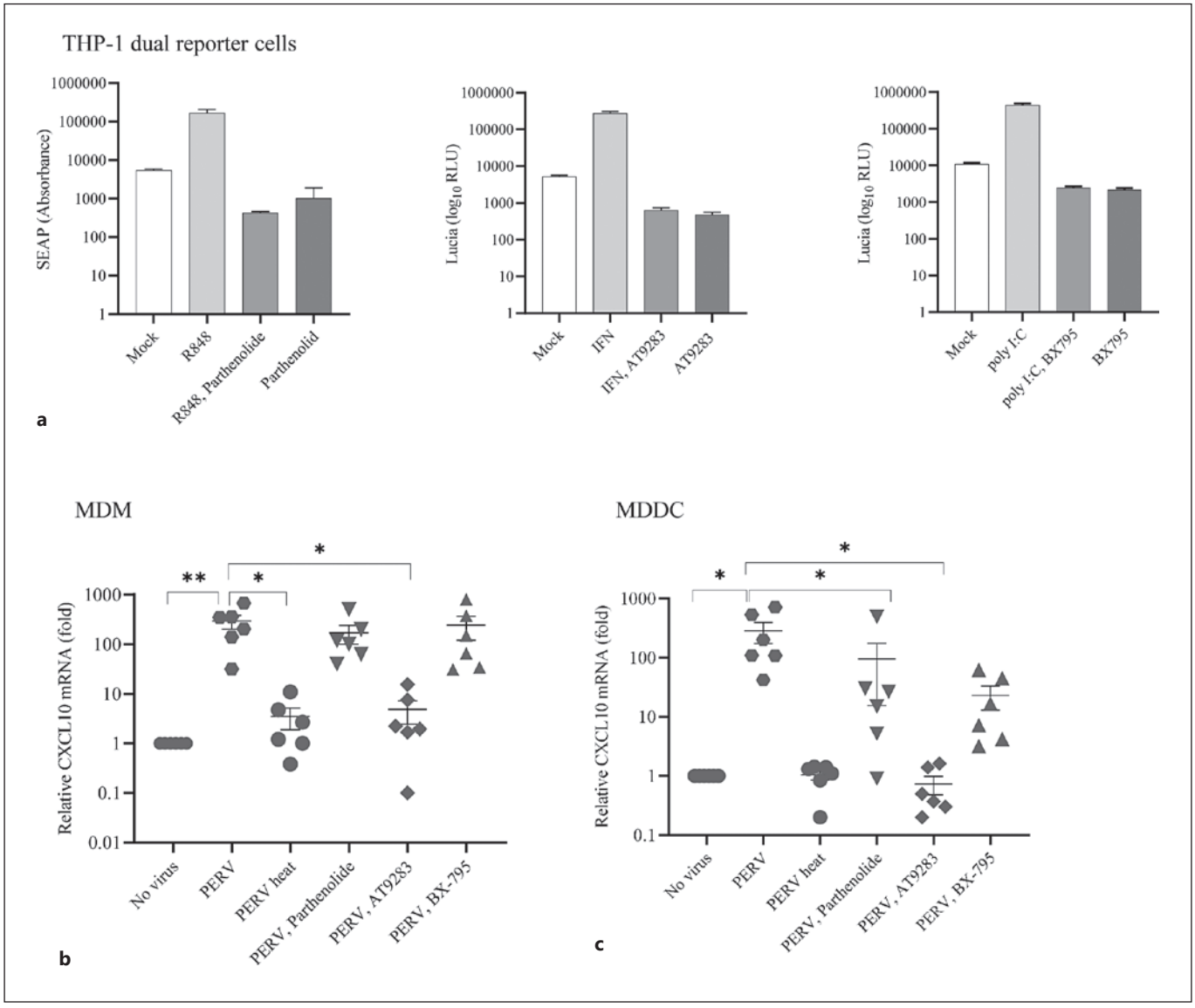

Fig. 5. The JAK-STAT pathway is involved in the CXCL10 response to PERV-A/C. **a** THP-1-Dual reporter cells were pre-treated with R848 (left panel), IFN-β (central panel and poly I:C (right panel) to activate innate immune signalling pathways, as indicated. Perthenolid, AT9283, and BX-795 were added as specified and SEAP or Lucia production measured 48 h later. Mean values of triplicates and SEM are shown. **b** MDM and (**c**) MDDC from 6 healthy donors were treated with the signalling pathway inhibitors and subsequently PERV-A/C was added, as indicted. Two days later, RNA was isolated and CXCL10 mRNA quantified relative to GAPDH. The results of cells not exposed to PERV-A/C were set to one and the ΔΔCT method was used to calculate the fold difference of CXCL10 mRNA to GAPDH. Mean values from triplicates +/− SD are shown. *$p < 0.05$; **$p < 0.005$; unpaired *t* test.

AZT does not inhibit PERV at a concentration of 10 μM [29, 30]. In addition, the integrase inhibitor RAL was used as further control. RAL inhibits PERV-A/C integration with an IC50 of 1 nM [32]. However, in our non-dividing primary cells integrase inhibitors should not modulate the outcome as PERV is a gammaretrovirus which preintegration complexes are unable to pass the nuclear poor. Therefore, infection cannot proceed up to the integration step in such cells.

The results obtained demonstrate that neither one of the two RT-inhibitors nor the integrase inhibitor had any effect on the CXCL10 induction in monocytes (Fig. 4a), MDM (Fig. 4b), or MDDC (Fig. 4c). Degradation of SAMHD1 did also not change the level of CXCL10 mRNA

in these cells. Usage of heat-inactivated PERV-A/C again prevented the CXCL10 increase. Taken together these results indicate that sensing of the virus that leads to the complete or almost complete CXCL10 induction occurs at an early replication step and does not require reverse transcription.

The JAK-STAT Pathway Is Crucial in the CXCL10 Response of Human Monocyte-Derived Cells to PERV-A/C

Several signal transduction pathways are known to be involved in the regulation of CXCL10 expression [33]. In order to identify the impact of presumed activation routes in monocyte-derived primary human cells, we have used inhibitors directed against three key players of major pathways. For inhibition of NF-κB the sesquiterpene lactone parthenolide was chosen [34]. For inhibition of the TANK-binding kinase 1 (TBK1), the aminopyrimidine compound BX-795 that also inhibits IκB kinase ε (IKKε) was added [35]. Janus kinases (JAKs) were inhibited with the compound AT9283 [36]. All inhibitors were dissolved in DMSO and used at a concentration of 1 μM. At this concentration, no toxic effects on the cells used were seen in the experimental setups.

To assess activity of these inhibitors, initial experiment with the monocyte model cell line THP-1 engineered as dual reporter cells were performed. These cells secrete alkaline phosphatase after stimulation of the NF-κB pathway and produce luciferase (Lucia) by activation of the IRF pathway. Following activation with the TLR7/8 agonist R848 (Resiquimod), a considerable increase in SEAP secretion has been measured. This increase could be completely prevented by addition of parthenolide (Fig. 5a). Furthermore, luciferase production was induced by adding both, IFN-β or poly I:C. As shown in Figure 5a, AT9283 prevented the IFN-β induced luciferase increase while BX-795 abrogated the effect following stimulation with poly I:C. These results demonstrate that all inhibitors used are biologically active.

To analyse the outcome of NF-κB, JAK, and TBK1 inhibition on the CXCL10 induction in MDM and MDDC, we have performed experiments with cells from six donors. The inhibitors were added 1 h before PERV-A/C was added and the level of CXCL10 induction was assessed 2 days later. In MDM, the JAK inhibitor AT9283 potently prevented the induction of CXCL10 by PERV-A/C in cells from all six donors. No inhibition was observed by treatment with parthenolide or BX-795 (Fig. 5b). In MDDC, AT9283 did also prevent activation. However, in MDDC from some of the donors, a partial inhibition of CXCL10 induction occurred by AT9283 and BX-795 as well (Fig. 5c).

Our results suggest that the JAK-STAT pathway is critically involved in the stimulation of CXCL10 expression in monocyte-derived primary human cells by PERV-A/C. Moreover, in some cells including MDDC, an involvement of additional pathways is evident.

Discussion

Monocytes and other related cells of the myeloid lineage are the major actors in innate immunity. It is therefore mandatory to focus on these cells when analysing innate immune activation following infection by PERV-A/C, a virus with zoonotic potential in the context of xenotransplantation. Our findings demonstrate that CXCL10 expression is strongly induced in human monocytes, MDM, and MDDC in response to a replication-competent PERV-A/C isolate. In relation to CXCL10, the stimulation of IFN-β and ISG54 2 days post virus encounter was very poor in most donors, at the best. This does not necessary mean that such a low induction could not have physiological consequences. A low expression and secretion of interferons might nevertheless be sufficient to act in an autocrine or paracrine fashion and stimulate only the most sensitive responses in a cell.

The strong and rapid CXCL10 induction by the retrovirus PERV-A/C we found is not without precedence. Viruses and especially retroviruses are known to elicit a robust upsurge of CXCL10 expression. CXCL10 mRNA is highly abundant in HIV-1 infected monocytes and MDDC and the expression level of this chemokine correlates with the viral load and disease progression [37]. In HIV patients, an increase in plasma serum levels and other body fluids has been documented as early as day 6 after the first positive PCR result which is a much faster response in comparison to 23 other cytokine levels analysed by Stacey and colleagues [38]. Furthermore, antiretroviral treatment diminishes plasma CXCL10 concentration but does not bring it down to levels in HIV negative individuals [23]. CXCL10 has also been proposed to serve as a potential marker for disease severity [23]. There is also evidence that the chemokine promotes HIV replication which is reasonable considering its pro-inflammatory activity [23, 39]. In this respect, the CXCL10 levels were found to be positively correlated with the probability for HIV infection [40]. Besides HIV, CXCL10 has also been reported to be induced by other retroviruses including polytropic murine retroviruses [41], EIAV [42], and

XMRV [43]. For XMRV, it has been demonstrated that recognition by the toll-like receptor 3 (TLR 3) plays a significant role for the induction of CXCL10 at the level of transcription [43]. Furthermore, the 10 kDa chemokine has been shown to be implicated in many other viral infections including rhinoviruses [44], Ebola [45], and SARS [46]. It reaches also high expression levels in the acute phase of Zika virus infection in monocytes of pregnant women [47].

Although the cellular restriction factor SAMHD1 inhibits PERV-A/C infection [27], its degradation in these cells did not change the responses in our analyses. SAMHD1 prevents reverse transcription by depleting the cytoplasmic dNTP pool. The lack of an increase of CXCL10 mRNA in cells with depleted SAMHD1 indicates that RT products are not the viral elements that are sensed by the cell and provoke the observed CXCL10 effect. This is corroborated by our results obtained with AZT, an RT-inhibitor for which PERV is highly susceptible [29, 30]. The prevailing CXCL10 induction in the presence of AZT argues against a significant role of PERV-A/C RT-products-sensing [48]. It is rather likely, that PERV-A/C PAMPs are sensed at an early replication step, presumably by TLRs in the endosome that serve as PRRs. This assumption is in line with the data we obtained using inhibitors of cellular signalling pathways. To demonstrate that the three inhibitors, we applied are active we used a monocytic reporter cell line. The data obtained subsequently with monocyte-derived cells provide evidence, that the JAK-STAT pathway is critically involved in the CXCL10 response. We regard it as likely, that this pathway is not directly connected to the PRR that is sensing the PERV-A/C PAMP. It could represent a secondary loop induced by interferons that amplify the initial innate immune response in an autocrine fashion and culminate in the strong CXCL10 induction we measure. This hypothesis deserves to be analysed more extensively in future work. It has to be mentioned in this respect that it is well established that CXCL10 is highly induced by type II and type I IFN mediated routes, but other signalling pathways are also involved [23, 37, 49]. Our findings regarding the signalling agrees with a previous work from Sakar and co-workers, showing that blocking of the JAK-STAT pathway by a JAK inhibitor almost completely inhibited CXCL10 production stimulated by respiratory syncytial virus infection while the NF-κB pathway appeared to be only slightly involved [50, 51].

In conclusion, our results provide evidence that the gammaretrovirus PERV-A/C induces a profound CXCL10 response in human monocytes and monocyte-derived primary cells at an early replication step that occurs before reverse transcription. A solid induction involves the JAK-STAT pathway. Although this and potentially other innate immune responses might be subdued by immunosuppressive drugs, it nevertheless remains a risk for the recipient of the xenotransplant. Thus, the elimination of infection competent PERVs and other pathogens from xenotransplants remains an important goal.

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Statement of Ethics

This study did not require ethical approval or consent in accordance with local/national guidelines. Primary cells used were derived from anonymous blood donations from the German Red Cross.

Conflict of Interest Statement

The authors declare that they have no competing interests and no conflict of interests exists.

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Author Contributions

Hussein Al-Shehabi and Norbert Bannert conceived and designed the study, analysed the data, and wrote the manuscript. Hussein Al-Shehabi performed all experiments.

Data Availability Statement

All data generated or analysed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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