Cytokine Effects on the Entry of Filovirus Envelope Pseudotyped Virus-Like Particles (VLP) into Primary Human Macrophages

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Abstract

Macrophages are among the first cells targeted in filovirus infection. In addition of being a major site of filovirus replication, macrophages also serve as a source of multiple cytokines, presumed to play a critical role in the pathogenesis of viral infection. By employing replication incompetent, filovirus GP1,2 pseudotyped, β-lactamase (BlaM)-containing VLPs, we have been able to assess the effects of specific cytokines, elevated during Ebola virus disease (EVD), on filovirus-cell fusion under BSL-2 conditions. We have previously established (Stantchev et al. Viruses 2019, 11, 889) that pre-incubation of primary human monocyte-derived macrophages (MDM) with interleukin-10 (IL-10) results in an increase of filovirus entry into these cells. To our surprise, we subsequently determined that not only IL-10, but also Type I and Type III interferon (IFN) pre-treatment of MDM, obtained from multiple healthy donors, significantly enhanced their ability to fuse with BlaM-containing VLPs, pseudotyped with the surface glycoprotein of all clinically relevant filovirus species. In contrast, and consistent with previously published studies, the IFNs potently reduced the entry of the same VLPs in the cell lines that were tested. Furthermore, the fusion and entry of influenza hemagglutinin (HA)/neuraminidase (NA) pseudotyped VLPs was suppressed by IFNs both in primary MDM and the specified cell lines. Our studies identified a novel and unexpected effect of Type I and Type III IFNs on filovirus GP-mediated fusion in primary human MDM and may provide insights for better understanding of Ebola virus pathogenesis. The acquired expertise may also be useful in establishing less hazardous systems for evaluation of potential new filovirus countermeasures.

Introduction

Interferons (IFN), especially Type I IFN, are integral and critical part of the antiviral innate immune responses. The ability of the Ebola virus (EBOV) to avoid these responses is well described and has been mainly attributed to the ability of the EBOV proteins p24 and p35 to antagonize the Type I IFN induced, signaling-mediated antiviral effects (reviewed in KÜhl and Pöhlmann 2012, Basler 2015, Bhattacharyya 2021). In general, IFNs are able to suppress virus infection both at the level of cellular entry and at post-entry virus replication steps. Inhibition of EBOV-cell fusion by interferon induced transmembrane proteins (IFITM) has been described in certain cell lines (Diamond and Farzan, 2013), but there are limited data regarding IFN effects on filovirus entry in one of their main targets, primary macrophages. To avoid the potential interference of any post-fusion factors and to study solely the effects of Type I and Type III IFNs on filovirus entry we employed BlaM) containing, filovirus GP pseudotyped VLP. As expected, IFN-α potently suppressed filovirus GP pseudotyped VLP fusion in the tested cell lines, but surprisingly enhanced the entry of the same VLP into primary human MDM. Consistent with a previous study using dendritic cells and primary monocytes (Perez-Zsolt et al. 2019) IFN-α induced Siglec-1 expression in MDM was also associated with increased EBOV GP pseudotyped VLP entry, but in contrast to these previously published results was not solely responsible for the observed effect. Investigations are currently ongoing to identify the additional factors potentially responsible for the enhanced filovirus GP pseudotyped VLP fusion with primary human MDM.

Materials and Methods

Cells. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-PaqueTM gradient centrifugation from the blood of healthy human donors. Monocytes and lymphocytes were further separated by countercurrent centrifugal cell elutriation. Macrophages were prepared from elutriated monocytes by differentiation for 7 to 14 days in DMEM supplemented with 10% pooled human serum , 2 mM L-glutamine and antibiotics (MØ medium).

VLP entry studies. BlaM or mCherry-containing VLPs were prepared by cotransfecting 293T cells with a plasmid encoding the relevant filovirus surface glycoprotein (GP) (provided by Dr. G. Nabel and Dr. A. Marzi)and a plasmid encoding the Ebola virus (EBOV) matrix protein VP-40 linked to the BlaM or mChery sequences (provided by Dr. L.Rong and Dr. J. White, respectively). VLP pseudotyped for the influenza hemagglutinin (HA) and Neuroaminidase (NA) (plasmids provided by Dr. C. Weiss) or vesicular stomatitis virus glycoprotein G (VSV-G) were generated using the psPAX2 packaging vector (Addgene, Dr. D. Trono) were employed as controls. After pre-incubation with the indicated cytokines [dissolved in DPBS, 0.5% human serum albumin (HSA)] for 48 h, the target cells were washed and allowed to fuse with the BlaM containing VLPs for 3 h-3.5 h in triplicate wells and loaded with CCF2/AM fluorescent dye for 1.5h. Subsequently the dye was removed and the cells were incubated overnight in phenol red free DM-10 at RT to allow cleavage of intracellular CCF2/AM by the virus introduced cytoplasmic BlaM. The cells were fixed with 1.6% paraformaldehyde and analyzed in situ using Laser Scanning Cytometer, equipped with violet (407 nm) laser and 460/40 and 530/25 nm emission filters. Successful cleavage of CCF2/AM by BlaM is detected by the change in the dye emission from the green (530nm) to the blue (460nm) spectrum. For Flow Cytometry (FC) cell were infected in triplicate wells, detached by Accutase or trypsin/EDTA, combined in one tube, fixed and analyzed. mCherry containing VLP were used to label control or cytokine treated cells at 4°C for 1h. The cells either washed and fixed to assess the VLP binding at the surface or incubated for 35 min at 37°C, treated with Trypsin/EDTA, fixed and analysed by Flow cytometry to evaluate VLP.

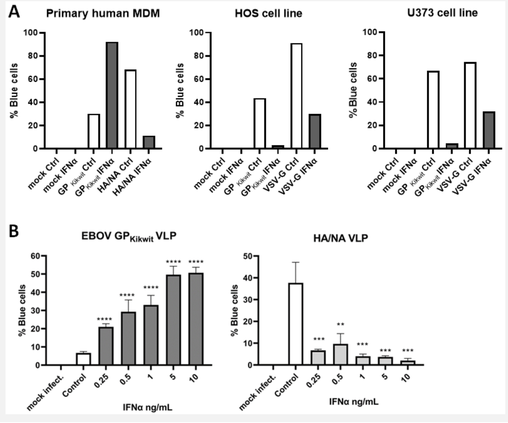

Figure 1. IFN-α enhances entry of EBOV GP_{Kikwit} pseudotyped VLP in primary human MDM, but suppressed VLP entry into cell lines. A. MDM, human osteosarcoma (HOS) or human glioblastoma U373 cell lines were treated with 0.5% HAS (control) or 10 ng/mL IFN-α for 48h, infected and analyzed by FC as described in M&M. **B.** MDM were incubated with different concentrations of IF-α,infected and analyzed *in situ* by Laser Scanning Cytometry. VSV-G or HA/NA pseudotyped VLP served as controls.

Results and Discussion

Type I and Type III IFN pre-incubation significantly enhance the entry into primary human MDM of VLP pseudotyped with the surface GP of Ebola (Kikwit, Mayinga or Makona strains), Sudan, Bundibugyo, Tai Forest and Marburg filoviruses (Figure 1, Fugure 2 and data not shown). In contrast, and consistent with previously published studies, type I IFNs potently reduced the fusion of the same VLPs into HOS, U373 and A549 cell lines (Figure 1, Fugure 2 and data not shown). The fusion of VLP pseudotyped with influenza HA/NA or VSV-G was inhibited in both primary MDM and the tested cell lines. The enhancement of filovirus GP pseudo-typed VLP entry into primary MDM was not associated with significant changes in NPC-1 and/or cathepsins levels, while the Type I and Type III IFNs-induced Siglec-1 (CD169) expression appeared at least partially responsible for this increase (Figure 3). Siglec-1 overexpression in U373 cells also increased EBOV GP_{Kikwit} VLP entry, but only partially reverse the inhibitory effect of IFN-α pre-incubation (Figure 4). This observation suggests the existence of additional factors responsible for the Type I and Type III IFN enhanced filovirus GP pseudotyped VLP entry into primary MDM.

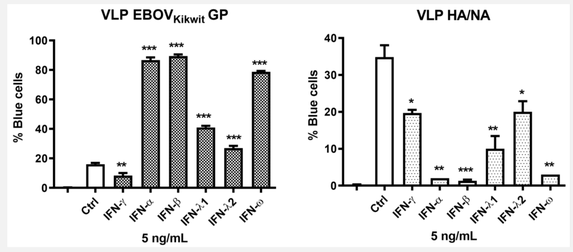

Figure 2. Type I and Type III interferons enhance entry of EBOV GP_{Kikwit} pseudotyped VLP in primary human MDM, but suppressed fusion of VLP pseudotyped with influenza HA/NA. Primary MDM were pre-incubated with cytokines for 48h and subsequently infected, processed and analyzed by Laser Scanning Cytometry.

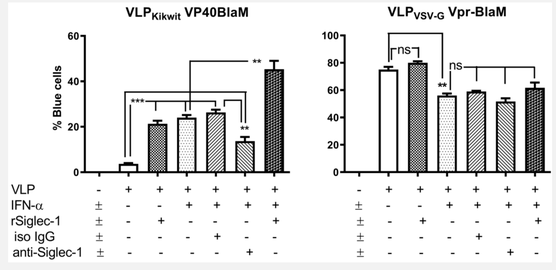

Figure 3. IFN-α and rSiglec-1 enhance entry of EBOV GP_{Kikwit} pseudotyped VLP into primary human MDM, while anti-Siglec-1 antibodies partially reverse the IFN-α effect. MDM were pre-incubated with IFN-α (5 ng/mL) for 48h and subsequently incubated with rSiglec-1 or polyclonal anti-Siglec-1 antibodies for 35 min. prior to infection. MDM were loaded with CCF2/AM and analyzed by Laser Scanning Cytometry.

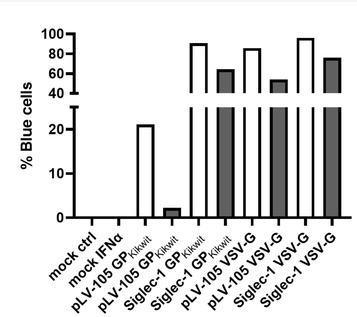

Figure 4. Overexpression of Siglec-1 significantly enhances EBOV GP_{Kikwit} pseudotyped VLP in U373 cells, but only partially reverse the inhibition by IFN-α pre-incubation.
Control and Siglec-1 encoding VLP, used to transduce U373 cells, were generated by cotransfection of 293T cells with the Genecopoeia pReceiver-LV105 control or pReceiver-LV105- Siglec-1 plasmid, psPAX2 and a VSV encoding plasmid. The control U373 cells were generated after puromycin selection and U373 cells stably overexpressing Siglec-1 were generated by puromycin selection and two rounds of cell sorting. The control (no fill columns) and Siglec-1 expressing cells (grey columns) were infected with EBOV GP_{Kikwit} pseudotyped VLP , processed and analyzed by FC as described in M&M. VSV-G pseudotyped BlaM containing VLP were used as a control.

Conclusion

1. Type I interferons have opposite effects on filovirus VLP entry in primary human MDM (enhancement) and cell lines (inhibition).
2. The cell-type specific difference is not due to changes in NCP-1, cathepsins and/or IFITM expression (data not shown)
3. The overexpression of Siglec-1 alone does not appear to be the cause for the observed differences in filovirus GP pseudotyped VLP entry established in primary human MDM and the tested cell lines.
4. Experiments with mCherry containing, EBOV GP_{Kikwit} pseudotyped VLP demonstrated that Siglec-1 overexpression augment VLP endocytosis in both primary human MDM and the tested cell lines (data not shown), suggesting that the cell type specific effects of IFN-α are likely at the level of VLP-endosomal membrane fusion
5. Based on our findings and previously published studies using authentic (wild type) viruses, it is likely that Type I and Type III interferons may have dichotomous effects at the level of virus entry (enhancement) and the subsequent post-fusion steps (inhibition) of the filovirus life-cycle in primary human MDM.