MPXV Replication Induces an IFN Response and Is Suppressed by IFN-γ

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BACKGROUND

Poxviruses employ different strategies to evade innate immunity, particularly the Interferon (IFN) response, but limited data are available for Monkeypox virus (MPXV). To address this knowledge gap and to study the interplay between MPXV infection and the IFN response, we investigated *in vitro* kinetics of IFN and the production of IFNs and Interferon stimulated genes (ISGs) in different anatomical districts of MPXV-infected patients and analyzed their correlation with MPXV DNA levels. In addition, we determined the *in vitro* anti-MPXV activity of IFN-α subtypes, IFN-β, IFN-ω and IFN-γ.

METHODS

Evaluation of MPXV growth curve replication and IFN induction following stimulation with MPXV and UV-inactivated VSV (MOI of 0.1 $TCID_{50}$/cell) was performed in human lung adenocarcinoma epithelial A549 cell line.

Twenty-one samples from different anatomical sites [Skin lesions (SL), anal canal brushings (ACB) and nasopharyngeal swabs (NSP), peripheral blood mononuclear cells (PBMC)] were collected from MPXV-infected male patients. Gene expression of type I (IFN-α/β/ω), type II (IFN-γ), type III IFNs (IFN-λ1/2/3) and ISGs (ISG15, ISG56 and PKR) was measured by quantitative RT-PCR assays. MPXV-DNA levels were evaluated using the Bioperfectus Monkeypox Virus Real Time PCR kit.

Antiviral assays were performed to assess the sensitivity of MPXV (MOI=0.1$TCID_{50}$/cell) to different types of IFNs (IFN-α, IFN-α natural, IFN-β, IFN-ω and IFN-γ) on different cell lines (A549 cells, cervival carcinoma HeLa cells and african green monkey kidney VeroE6 cells).

MPXV delays IFN-I induction and is more sensitive to IFN-γ than to IFN-I

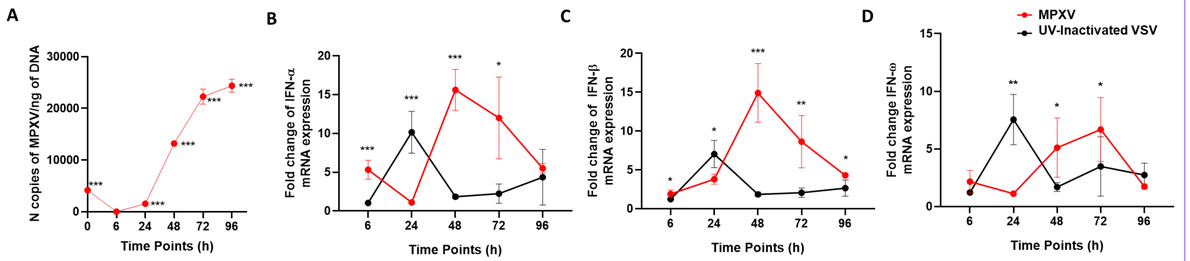
RESULTS

The kinetic of MPXV replication in A549 cells showed a reduction of MPXV titer by approximately 2 Log at 6h post infection (p.i.) compared to the inoculum, while the titer started to increase at 24h p.i. with a peak between 48 and 72 h p.i. ($p<0.001$) (Fig. 1, Panel A). MPXV stimulated a delayed production of type I IFNs (IFN-α, IFN-β, IFN-ω) with a peak at 48h for IFN-α and between 48h and 72h for IFN-β and IFN-ω ($p<0.05$) compared to UV-inactivated VSV stimulation where IFNs peaked at 24 h (Fig. 1, Panels B-D).

Differential IFN-I, IFN-II, IFN-III and ISGs transcript expression was observed in skin lesions, anal canal brushings, nasopharyngeal swabs and peripheral blood mononuclear cells. IFN-α and IFN-λ1 were upregulated in anal canal brushings compared to the other clinical samples ($p<0.05$), and IFN-β, IFN-ω and IFN-γ mRNA levels were increased in skin lesions ($p<0.05$). Transcript levels of ISG56 and IDO were higher in nasopharyngeal swabs ($p<0.05$), while PKR was more expressed in anal canal brushings ($p<0.001$). A significant negative correlation was found between MPXV DNA Ct values and IFN-α, IFN-β and IFN-ω (Fig. 2, panels A-B-C). In addition, although not statistically significant, a trend toward an inverse relationship was observed between IFN-γ mRNA levels and MPXV DNA Ct values (Fig. 2, panel D).

We investigated the sensitivity of MPXV to the antiviral activity of the IFN-α subtypes, IFN-β, IFN-ω and IFN-γ (Fig. 3). Pre-treatment of A549 with high concentration of IFN-α2 (500 000 IU/mL) and IFN-α subtypes contained in the natural IFN-α preparation (300 000 IU/mL) caused a slight decrease in MPXV titer ($p<0.001$). Treatment with IFN-ω at the highest concentration (300 000 IU/mL) reduced MPXV titers by 1.37 Log ($p<0.001$), with no effect on virus replication at concentrations ≤1360 IU/mL. By contrast, IFN-β was more effective than the other type I IFNs, with treatment using 100 000 IU/mL resulting in a 2.75 Log reduction in MPXV titer down to 450 IU/mL, which reduced the viral titer by 0.37 Log ($p<0.001$). IFN-γ treatment showed the strongest antiviral effect. Specifically, treatment with 3600 IU/mL reduced viral titer by 3.37 Log and the reduction was ≥2.75 down to 400 IU/mL ($p<0.001$). Results from the Hela and Vero-E6 cell lines confirmed a general trend showing a greater anti-MPXV activity of IFN-β compared to other IFN-I, and the strongest antiviral effects of IFN-γ against MPXV.

Fig. 1



*p<0.05; **p<0.01; ***p<0.001

Fig. 2

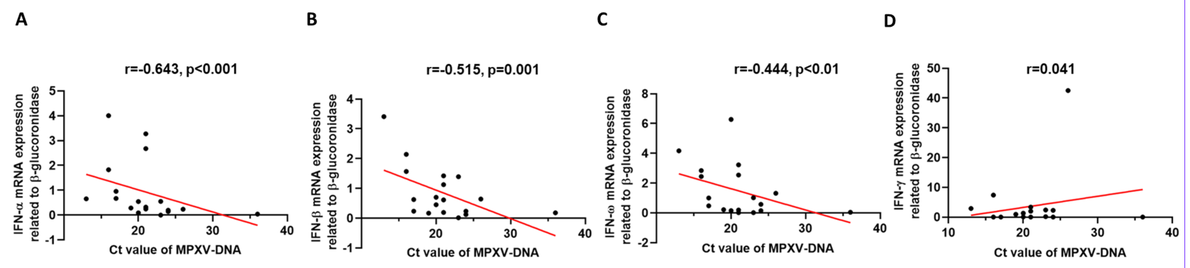
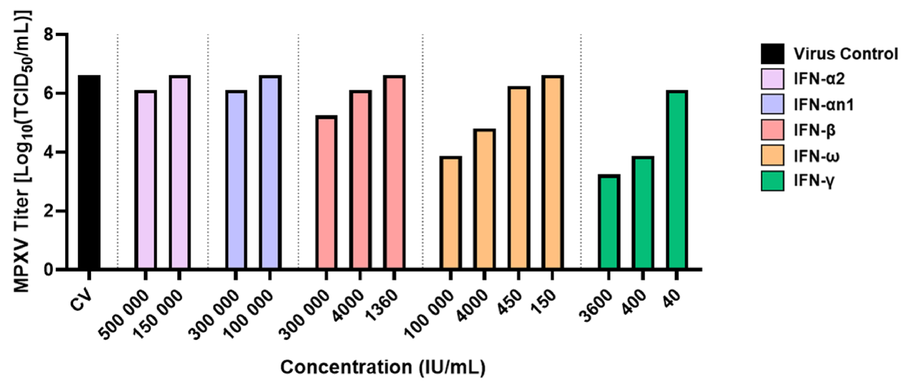


Fig. 3



CONCLUSIONS

This study provides evidence that type I IFN are produced but are significantly delayed after MPXV infection. Type I IFNs induction after MPXV infection was not associated with a decrease in the viral titer in vitro and ex vivo. Consistent with these observations, we found that pre-treatment of A549, Hela and Vero-E6 cell lines with IFN-α and IFN-ω did not significantly inhibit MPXV replication. However, IFN-β and IFN-γ showed moderate and strong effects against MPXV replication, respectively.