Differential HLA-E Upregulation during JC Polyomavirus Infection Across Human Cell Types

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Background

- Progressive multifocal leukoencephalopathy (PML) is a rare, often fatal demyelinating disease caused by JC polyomavirus (JCPyV) reactivation in immunocompromised individuals.
- JCPyV may evade immune responses by upregulating HLA-E, a non-classical MHC class I molecule that interacts with inhibitory NK cell receptors, thereby reducing antiviral immunity.

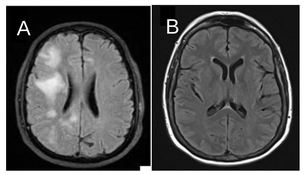

Figure 1: MRI of a classic PML patient with multiple lesions demyelination (A) and a case study patient with no apparent lesions (B).

Introduction

- Prior studies have shown HLA-E upregulation in JCPyV-infected glial cells.
- This study evaluates whether HLA-E expression increases alongside viral antigen levels during different stages of JCPyV infection and natural killers (NK) cell co-culture across multiple human cell types, aiming to clarify immune evasion mechanisms that contribute to PML.

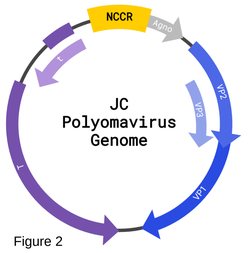

Figure 2

Methods

A Human cells were cultured in their respective media at 37 °C with 5 % CO_2 to 80–90 % confluency, then seeded at 10,000 cells per well in 96-well plates and infected with either JCPyV TURBO or MAD1 strains.

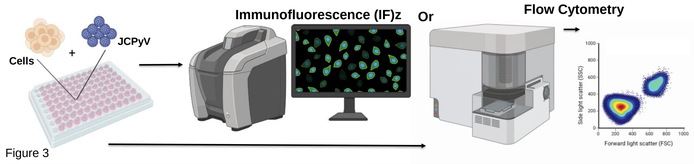

Figure 3

Cell Types	JCPyV strain	MOIs	Days Post-infection
SVG-A (glial)	TURBO	0.3, 0.6, 1.25, 2.5, 5	Flow Cytometry at 10 dpi, and IF 14 dpi
HEK 293 FT (kidney)	TURBO	0.015, 0.03, 0.06, 0.125, 0.25	Flow Cytometry at 11 dpi
Monocytes and Macrophages	MAD1	5	Flow Cytometry at 5 dpi

Analysis: Marker expression (HLA-E, VP1, and T antigen) was quantified by flow cytometry using the Cytek Aurora system and subsequently analyzed with FlowJo software, while VP1 and T antigen were also visualized by immunofluorescence using a Keyence BZ-X fluorescence microscope at 4x magnification with DAPI nuclear staining.

B Human NK cells were isolated from PBMCs using magnetic bead separation.

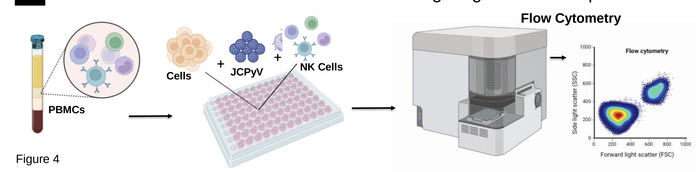

Figure 4

Cell Types	JPCyV strain	MOIs	Co-culture with NK cells	Days post-infection
Monocytes and Macrophages	MAD1	5	6 hours	Flow Cytometry at 5 dpi
SVA-A (glial)	TURBO	1	6 hours	Flow Cytometry at 10 dpi

Analysis: Marker expression (HLA-E, VP1, and T antigen) was quantified by flow cytometry using the Cytek Aurora system and subsequently analyzed with FlowJo software.

Results

VP1 and T Antigen Expression in SVG-A Cells

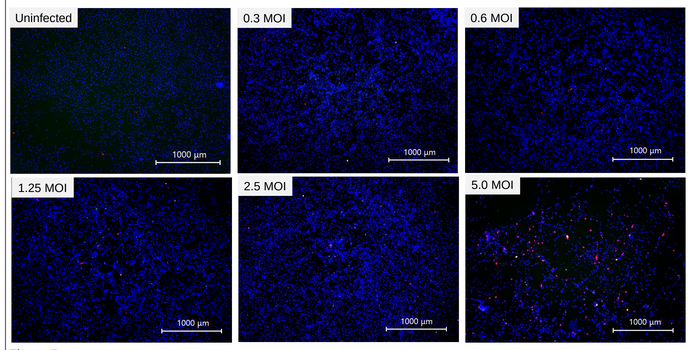

Figure 5

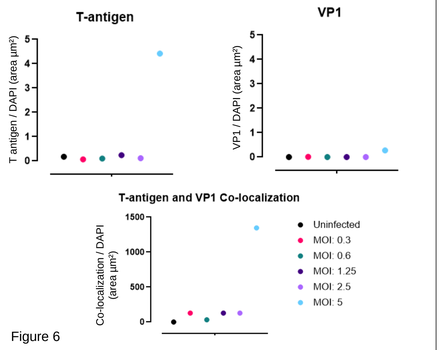

Figure 6

Figures 5–6: Immunofluorescence images of JCPyV-infected SVG-A cells at 14 days post-infection (dpi) stained with DAPI (blue), VP1 (green), and T-antigen (red). Increasing MOIs are shown from left to right. Quantification of VP1, T-antigen, and co-localization area (Figure 6) was performed using Fiji/ImageJ macro-based analysis and plotted using GraphPad Prism.

HLA-E Expression and JCPyV Infection Across Human Cell Lines

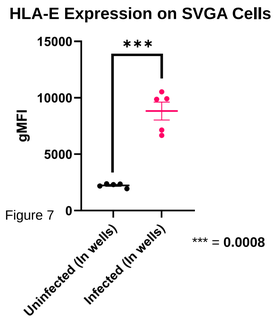

Figure 7

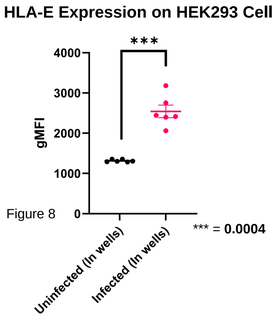

Figure 8

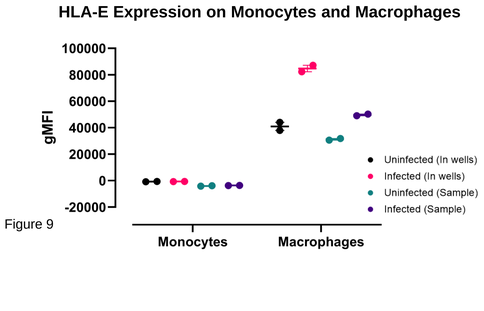

Figure 9

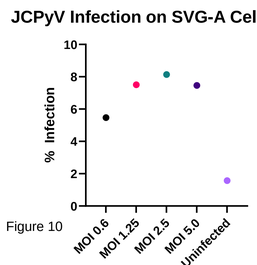

Figure 10

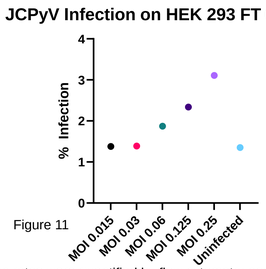

Figure 11

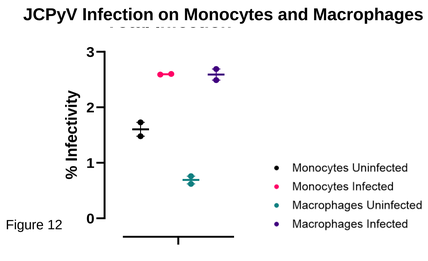

Figure 12

Figures 5–10: HLA-E expression and JCPyV infection rates were quantified by flow cytometry across different human cell types, including SVG-A, HEK 293 FT, monocytes, and macrophages. Data were analyzed with FlowJo and plotted using GraphPad Prism. One-way ANOVA with multiple comparisons was performed for Figures 7 and 8.

HLA-E Expression on JCPyV-Infected Cells Co-culture with NK

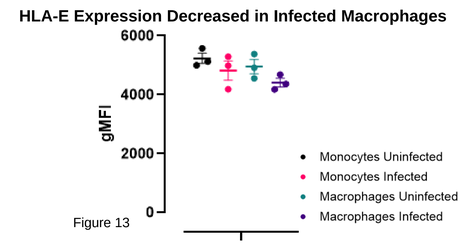

Figure 13

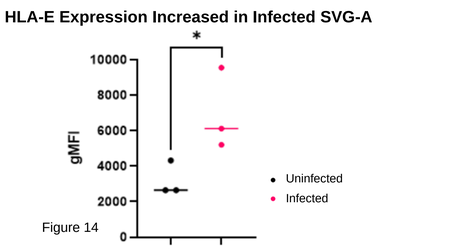

Figure 14

Figures 13–14: HLA-E expression and JCPyV infection were measured by flow cytometry in monocytes, macrophages, and SVG-A cells after NK co-culture. Data were analyzed with FlowJo and plotted in GraphPad Prism. ANOVA was applied to SVG-A (Fig. 14).

Conclusions

- JCPyV infection increased with MOI across cell types; however, in SVG-A cells, flow cytometry revealed a slight decrease at 5 MOI, possibly due to cytopathic effects, reduced viability, or infection saturation.
- HLA-E expression was significantly upregulated after infection in both SVG-A and HEK 293 FT cells, and increased in infected monocytes and macrophages, with macrophages showing moderately elevated baseline levels of HLA-E.
- NK cell co-culture affected HLA-E expression differently across cell types: it increased HLA-E in infected SVG-A cells, but reduced HLA-E in infected macrophages, suggesting that NK cells may influence HLA-E expression in a cell-type specific manner that could reflect differences in immune recognition or susceptibility.
- Together, these findings indicate that JCPyV modulates HLA-E expression in ways that depend on both the cell type and the presence of immune interactions, which may have implications for how infected cells are detected or regulated by the immune system, particularly in the context of diseases like PML.

Future Directions

- Repeat infection and HLA-E expression experiments in HEK 293 FT cells using higher MOIs.
- Expanding NK co-culture experiments to additional cell types (HEK 293 FT).
- Analyze both infection markers (VP1, T antigen) and HLA-E expression over multiple timepoints post-infection (e.g., days 3, 5, 7,14).
- Apply HLA-E blocking antibodies in infected cells to determine whether reducing HLA-E restores NK cell cytotoxicity or increases viral clearance.

References

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