Murine gammaherpesvirus 68 efficiently infects myeloid cells resulting in atypical, restricted form of viral infection

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Introduction

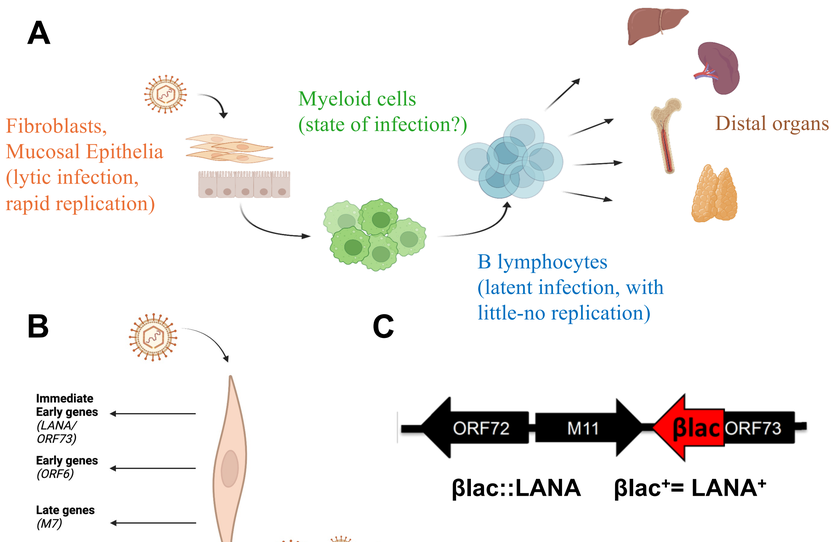
The human gamma herpesviruses (γHV) are viruses that cause lifelong infection, are found in approximately 95% of adults worldwide, and in conditions of immune deficiency, are associated with inflammation and oncogenesis. The human γHVs include:

- Kaposi's sarcoma associated herpesvirus (KSHV), and
- Epstein-Barr virus (EBV).

Murine gammaherpesvirus 68 (MHV68) is a mouse model of γHV infection and pathogenesis that provides new insights γHV infection.

While myeloid cells are known to be an early target of MHV68 infection in vivo, little is known about the nature and outcome of this infection.

Background



Characteristics of MHV68 infection (A) Pathway of MHV68 infection. Infection begins in fibroblasts/mucosal epithelia with lytic replication, followed by poorly characterized infection of myeloid cells, culminating in lifelong latent infection of B lymphocytes and systemic infection. **(B)** Schematic of canonical MHV68 lytic infection known to occur in fibroblasts. After entry, immediate early genes (transactivators) are produced followed by early genes (DNA synthesis/nucleotide metabolism) and late genes (structural components). **(C)** The MHV68 ORF73 gene encodes for the latency-associated nuclear antigen (LANA) protein, expressed throughout all stages of infection. We use an MHV68 reporter virus to identify infected cells, in which the ORF73 gene is fused to beta-lactamase, allowing for fluorescent detection of infected cells.

1. Impaired viral DNA replication despite efficient in vitro infection of a macrophage cell line

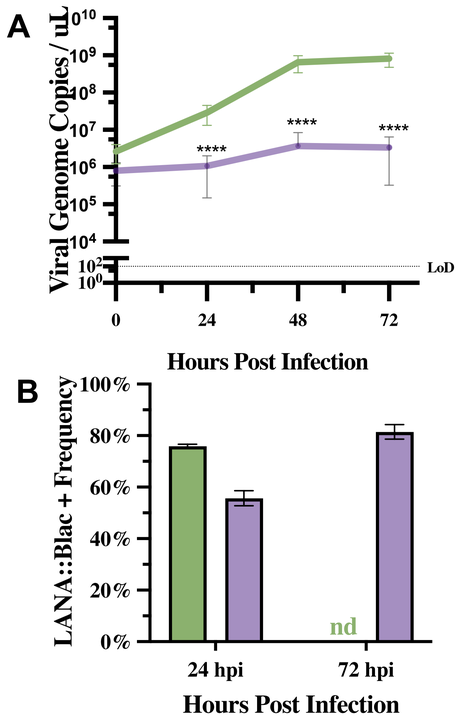


Figure 1: MHV68 infection of the J774 macrophage cell line demonstrates impaired viral DNA replication despite comparable infection efficiency to 3T12 fibroblasts. (A) Mean number of viral genome copies was quantified at 24, 48, and 72 hours post infection (hpi) by qPCR targeting the viral gB gene. MHV68 undergoes lytic replication in 3T12 fibroblasts (in green), a condition resulting in a 2.5 log increase in viral genome copies/uL which is not observed in J774 cells. (n=3) **(B)** Mean frequency of LANA::Blac+ cells detected by flow cytometry at 24 and 72 hpi. MHV68 infection kills 3T12 fibroblasts by 72hpi (n=3).

3T12 Fibroblast line
J774 Macrophage line

2. Impaired MHV68 replication, gene expression in infected macrophages

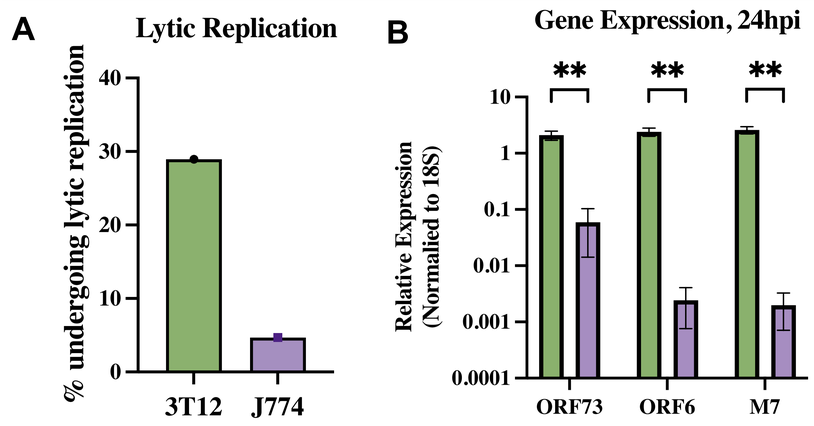


Figure 2: Lytic replication and gene expression of γHV68 is defective at 24 hpi in J774 myeloid-like cell line. A) Quantification of MHV68 lytic replication, defined by flow cytometry-based detection of two lytic-replication associated proteins, vRCA (a viral late protein) and γH2AX (cellular protein modified by viral infection) at 24 hpi. Mean values show29% 3T12 and 5% of J774 cells undergo lytic replication. (n=3) **B)** qRT-PCR for ORF73, ORF6, and M7 (immediate early, early and late genes, respectively) comparing lytic replication (3T12s) vs. restricted (J774s). Mean of three replicated shown (n=3)

3. Atypical gene expression of ORF75 in MHV68-infected J774 cells in vitro

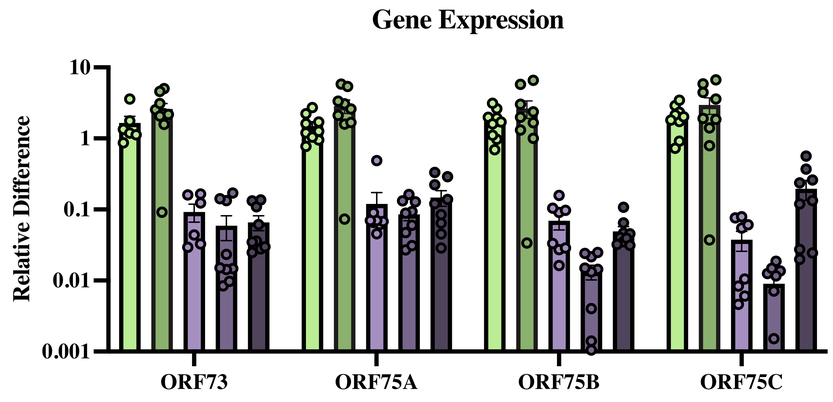
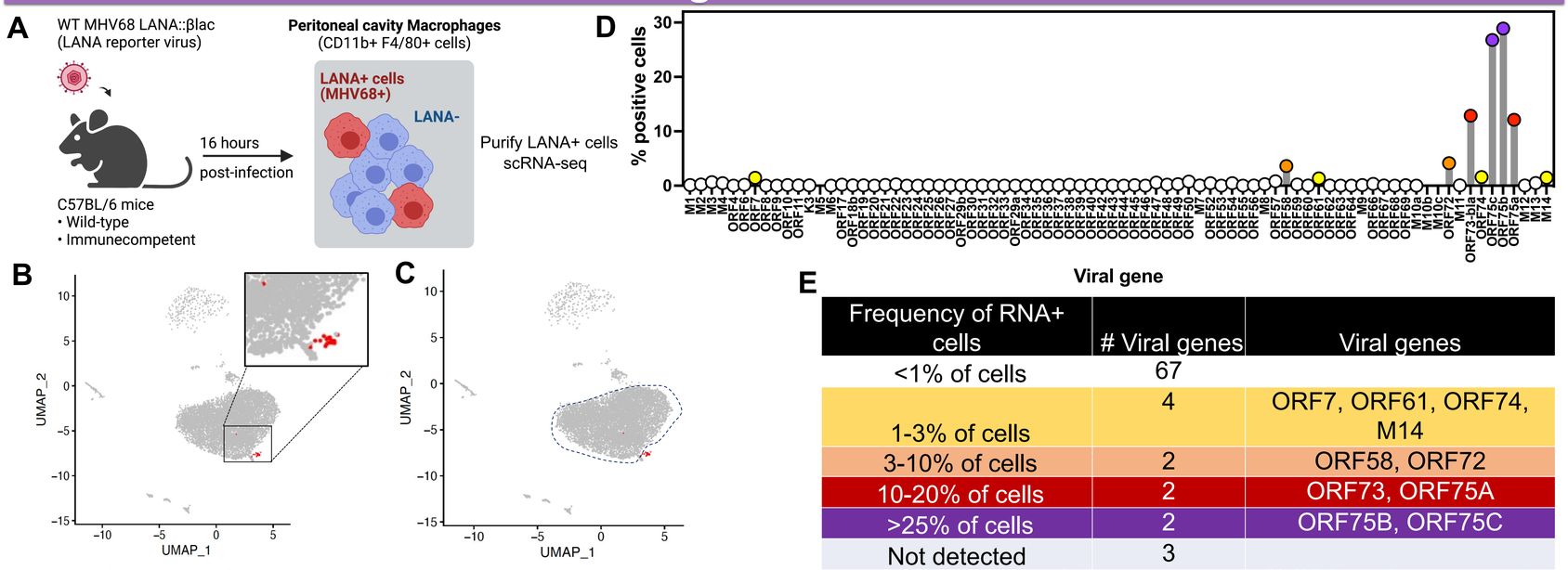


Figure 3: MHV68 infection of J774 cells is characterized by relatively high expression of ORF75A, 75B and 75C compared to ORF73. MHV68 gene expression in infected 3T12 or J774 cells followed by qRT-PCR. While ORF75 expression was expected to be low given defective lytic replication and viral gene expression in MHV68-infected J774 cells, these studies demonstrate unexpectedly robust ORF75 compared to ORF73 in infected J774 cells. Mean values shown, n=3.

4. Atypical gene expression of MHV68 in peritoneal macrophages purified following in vivo infection



Frequency of RNA+ cells	# Viral genes	Viral genes
<1% of cells	67	
1-3% of cells	4	ORF7, ORF61, ORF74, M14
3-10% of cells	2	ORF58, ORF72
10-20% of cells	2	ORF73, ORF75A
>25% of cells	2	ORF75B, ORF75C
Not detected	3	

Figure 4: Single-cell RNA sequencing of MHV68 infected peritoneal macrophages reveals distinct transcriptional outcomes. **A)** Wild-type immunocompetent C57BL/6 mice were infected via intraperitoneal injection with WT MHV68 LANA::Blac virus, with LANA+ MHV68-infected peritoneal cavity macrophages collected at 16 hpi. **B,C)** UMAP-based visualization of scRNA-seq data from LANA+ peritoneal macrophages reveals: **(B)** rare macrophages with lytic replication (expression of 15+ viral genes, red) and **(C)** a large fraction of infected cells with limited viral RNA expression (blue dotted circle, non-lytic population). **(D-E)** Percentage of LANA+ peritoneal macrophages that express each viral gene identifies a high frequency of ORF75B and ORF75C expression, that exceeds ORF73 expression.

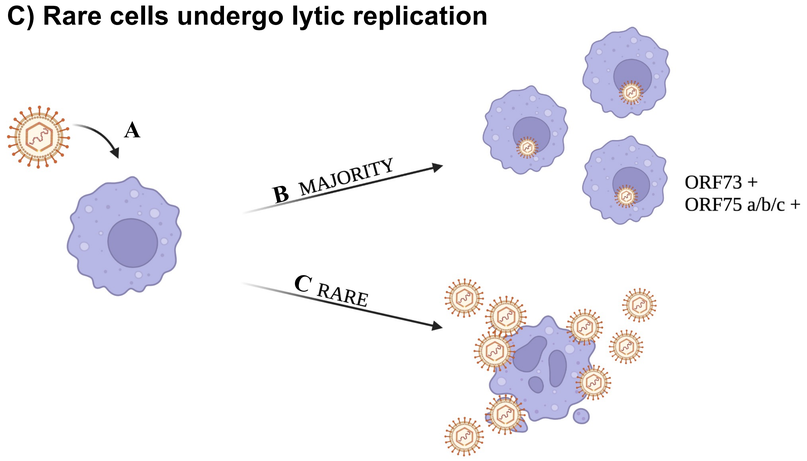
Conclusions

MHV68 infection of macrophages (the J774 cell line and primary cells) reveals:

A) Efficient viral entry and immediate early gene expression,

B) Majority of cells → Limited viral RNAs (ORF73,ORF75)

C) Rare cells undergo lytic replication



Future Directions

- What is the nature of infection in myeloid cells - latent, lytic, or a previously undescribed alternative outcome?
- What allows some rare macrophages to undergo lytic infection?
- Why is ORF75 expression atypical in myeloid cell infection? Functional consequences? Marker of alternate infection?

Acknowledgments

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