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Murine gammaherpesvirus targets type I IFN receptor but not type III IFN receptor early in infection

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ABSTRACT

The innate immune response represents a primary line of defense against invading viral pathogens. Since epithelial cells are the primary site of gammaherpesvirus replication during infection *in vivo* and there are no information on activity of IFN-III signaling against gammaherpesviruses in this cell type, in present study, we evaluated the expression profile and virus-host interactions in mouse mammary epithelial cell (NMuMG) infected with three strains of murine gammaherpesvirus, MHV-68, MHV-72 and MHV-4556. Studying three strains of murine gammaherpesvirus, which differ in nucleotide sequence of some structural and non-structural genes, allowed us to compare the strain-dependent interactions with host organism. Our results clearly demonstrate that: (i) MHV-68, MHV-72 and MHV-4556 differentially interact with intracellular signaling and dysregulate IFN signal transduction; (ii) MHV-68, MHV-72 and MHV-4556 degrade type I IFN receptor in very early stages of infection (2–4 hpi), but not type III IFN receptor; (iii) type III IFN signaling might play a key role in antiviral defense of epithelial cells in early stages of murine gammaherpesvirus replication; (iv) NMuMG cells are an appropriate model for study of not only type I IFN signaling, but also type III IFN signaling pathway. These findings are important for better understanding of individual virus-host interactions in lytic as well as in persistent gammaherpesvirus replication and help us to elucidate IFN-III function in early events of virus infection.

1. Introduction

The innate immune system represents the first line of host defense during virus infection. Intracellular pattern recognition receptors are responsible for recognition of viral double-stranded and single-stranded RNA or DNA. Activated receptors induce signal transduction and phosphorylation of several transcription factors, which transactivate a large number of antiviral proteins, including interferons (IFNs). In general, the expression of IFNs is triggered mainly by three transcription factors; interferon regulatory factor-3 (IRF-3), IRF-7 and nuclear factor-kappaB (NF-κB) [1,2].

IFNs are a large group of cytokines, possessing a wide range of biological activities and serve as a first barrier in the defense against pathogen infection and malignant proliferation. Binding of IFNs to specific surface receptor activates the Janus kinase/signal transducers and activators of transcription (Jak/STAT) signaling pathway, leading to the expression of interferon-stimulated genes (ISGs) that mount an antiviral response and protect surrounding cells against virus infection [3]. However, the expression of ISGs can also be induced directly by transcription factors that are activated by virus infection [4]. Three classes of IFNs were described in humans, type I IFNs (IFNs-I), which include IFN-α, -β, -ω, -ε and IFN-κ; type II IFN (IFN-II) with one member, IFN-γ; and type III IFNs (IFNs-III, known as IFNs-λ), which include IFN-λ1, -λ2, -λ3, and recently discovered IFN-λ4 [5,6]. All three classes of IFNs

Abbreviations: EBV, Epstein-Barr virus; FBS, fetal bovine serum; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HHV-4, human herpesvirus 4; HHV-8, human herpesvirus 8; hpi, hours post infection; IFN, interferon; IFNAR, interferon α/β receptor; IFN-λR, interferon lambda receptor; IRF-3, interferon regulatory factor 3; IRF-7, interferon regulatory factor-7; ISG, interferon-stimulated gene; Jak/STAT, Janus kinase/signal transducers and activators of transcription; KSHV, Kaposi's sarcoma-associated herpesvirus; KS, Kaposi's Sarcoma; MAVS, mitochondrial antiviral signaling; MHV-68, murine gammaherpesvirus 68; MHV-72, murine gammaherpesvirus 72; MHV-4556, murine gammaherpesvirus 4556; MOI, multiplicity of infection; MuHV-4, murid herpesvirus 4; Rta, replication and transcription activator; VSV, vesicular stomatitis virus; MOI, multiplicity of infection; OAS-1, oligoadenylate synthetase 1; PKR, protein kinase R; TBK1, tank binding kinase 1.

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bind to specific surface receptors. IFNs-I bind to the interferon-α/β receptor (IFNAR), composed of two subunits referred to as IFNAR1 and IFNAR2. Interferon lambda receptor (IFNLR), which is composed of two subunits referred to as IFN-λR1 and IL10R2, binds IFNs-λ [7]. The IFNs-I are used in the treatment of several cancers, but the therapy is usually associated with high tissue toxicity and inflammatory symptoms, responsible for the hematological and neurological side effects [8]. Due to cell-type specific expression of receptor subunit IFN-λR1, the activation of IFN-λ signaling occurs in a more specific subset of cells, including epithelial cells [9], hepatocytes [10], and the cells of immune system such as naïve and memory human $CD4^+$ T cells [11], monocytes and macrophages [12]. That is the reason why IFNs-λ have a great potential to be used for treatment of several viral diseases, including cancers and diseases caused by herpesviruses [13–17].

Two human gammaherpesviruses, Epstein-Barr virus (EBV, known as Human herpesvirus 4 (HHV-4)) and Kaposi's sarcoma-associated herpesvirus (KSHV, known as HHV-8), are associated with several types of malignant diseases [18]. These include Burkitt's lymphoma and nasopharyngeal carcinoma (both associated only with EBV), classic Hodgkin's lymphoma, post-transplant lymphoproliferative disorders, HIV-associated lymphoproliferative disorders, several non-Hodgkin lymphomas and Natural killer cell and T cell lymphomas [19–21]. Kaposi's Sarcoma (KS) caused by KSHV is the second most frequent tumor in patients with AIDS [22]. However, due to strict host specificity of EBV and KSHV, Murid herpesvirus 4 (MuHV-4) alias Murine gammaherpesvirus isolate 68 (MHV-68), which is closely related to EBV and KSHV, represents a valuable tool for understanding the interactions between gammaherpesviruses and their hosts [23]. The pathogenesis of MHV-68 is similar to that of EBV and KSHV. The primary site of MHV-68 replication are epithelial cells of respiratory tract and lung. MHV-68 can establish latency mainly in B cells of germinal centers in spleen, in bone marrow, thymus, dendritic cells and peritoneal macrophages [24,25]. Persistent infection of MHV-68 can lead to development of lymphoproliferative diseases. It was found that approximately 9% of mice develop lymphomas associated with both lymphoid and nonlymphoid tissues [26].

In addition to prototype MHV-68, seven murine gammaherpesviruses known as -60, -72, -76, -78, -4556, -5682, and -Šumava were isolated from small wild-living rodents in Slovakia and the Czech Republic [27,28]. Generally, all isolates/strains are very similar, nevertheless, they differ from each other in some pathogenic properties related to differences in primary sequence of several genes as well as in a variability of the left end of their genomes. It was reported that the strain MHV-72 [29] is able to replicate effectively in the mammary glands of female nu/nu Balb/c mice and to transmit to the offspring via breast milk [30]. Recent results obtained in our study on biological properties of MHV-72 and MHV-4556 showed reduced ability of these two strains to establish latency in spleen and to reactivate *ex vivo* from latency in lung, spleen and thymus in comparison to MHV-68, when reactivation of latent virus was stimulated with the inhibitor of histone deacetylases, trichostatin A. Moreover, the whole genome sequencing of MHV-72 and MHV-4556 revealed deletion in the left end of their genomes, causing the absence of genes vt-RNAs, M1, M2, and confirmed previously identified mutations in several genes (Režuchová et al., unpublished data) [31,32], which could lead to distinct functionality of corresponding proteins, and thus account to distinct host-pathogen interactions.

Despite the intensive study of interactions between gammaherpesvirus and the host, the IFN-λ signaling network after murine gammaherpesvirus infection *in vitro* as well as *in vivo* has not been characterized yet. Here, we report the expression profile of IFN-λ, IFN-α and IFN-β, subunits of type III IFN receptor (IFN-λR1, IL-10RB) and type I IFN receptor (IFNAR1, IFNAR2) and specific ISGs in early stages of lytic replication of MHV-68, MHV-72 and MHV-4556 in epithelial cells NMuMG. Our results demonstrate that MHV-68, MHV-72 and MHV-4556 differentially interact with intracellular signaling and dysregulate IFN signal transduction. Very early infection of NMuMG cells with MHV-68, MHV-72 and MHV-4556 (2–4 hpi) leads to degradation of type I IFN receptor, but not type III IFN receptor. The expression of both type III IFN receptor and IFN-λ in early stages of infection suggests that type III IFN signaling might play a key role in antiviral defense during early gammaherpesvirus replication in epithelial cells. Finally, we clearly demonstrated, that NMuMG cells are an appropriate model for study of not only type I IFN signaling, but also type III IFN signaling pathway.

2. Materials and methods

2.1. Cell lines and viruses

The NMuMG mouse mammary epithelial cell line (ATCC® CRL-1636™) and the RAW 264.7 mouse macrophage, Abelson murine leukemia virus transformed cell line (ATCC® TIB-71™) were grown in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich) supplemented with 5% (v/v) fetal bovine serum (HyClone), 2 mM L-glutamine, 100 U of streptomycin per ml and 100 U of penicillin per ml (Lonza) and incubated at 37 °C in 5% CO_2 atmosphere.

Experiments were performed with three isolates of MuHV-4: MHV-68 clone f2.6, MHV-72 clone h3.7 [29] and MHV-4556 clone i2.8 [33]. Working virus stocks were prepared and titered on BHK-21 cells at the multiplicity of infection (MOI) of 0.05 PFU/cell. Vesicular stomatitis virus (VSV, Indiana strain) was prepared and titered on Vero cells at the MOI of 0.1 PFU/cell. The virus stocks were stored at −80 °C until further use.

2.2. Single-step growth curve and plaque assay

NMuMG cells were seeded into 24-well plate plate (1×10^5 cells/well) and cultivated in DMEM supplemented with 5% FBS at 37 °C in 5% CO_2 overnight. NMuMG cells were infected with MHV-68, MHV-72 or MHV-4556 at MOI of 5. The cells were washed two times with physiological saline (pH - 7.2) 15 min post infection and incubated in 1 ml of fresh DMEM supplemented with 5% FBS at 37 °C in 5% CO_2. The cells were harvested at different time points (0, 6, 8, 12, 16, 20, 24, 30, 40, 48, 60, 72 h post infection (hpi)), lysed by freeze-thaw cycle and the virus titer was determined by plaque assay. Briefly, NMuMG cells were seeded into 24-well plate (1×10^5 cells/well). The next day the cells were incubated with 100 µl serial dilution of the samples. After 1 h, the cells were washed two-times with physiological saline (pH - 7.2) and 1 ml of DMEM containing 0.43% methylcellulose was added, and the plates were further incubated for 96 h at 37 °C in 5% CO_2. Cells were subsequently stained with 0.03% methylene blue to allow quantification of plaques. All samples were tested in duplicate.

2.3. Infection of cells and RNA isolation

NMuMG cells and RAW 264.7 cells were seeded into 24-well plate at a concentration of 1×10^5 cells/well and cultivated for 24 h at 37 °C in 5% CO_2. NMuMG cells were infected with MHV-68, MHV-72 or MHV-4556 at MOI of 1 PFU/cell. In addition, NMuMG cells and RAW 264.7 cells were infected with VSV at MOI of 1, which served as a control for cytokine expression. To extract total RNA, infected cells were washed two times with physiological saline (pH - 7.2) and lysed with Buffer RLT Plus (Qiagen) at 0, 2, 3, 4, 5 and 6 hpi. Total RNA was isolated using