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MxA-independent inhibition of Hantaan virus replication induced by type I and type II interferon in vitro

Robin Oelschlegel, Detlev H. Krüger, Andreas Rang *

Institute of Virology, Helmut-Ruska-Haus, University Hospital Charité, Charité Campus Mitte, D-10098 Berlin, Germany

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Abstract

Interferons (IFN) induce an antiviral state against Hantaan virus (HTNV) but the mechanisms responsible for inhibition are unclear. The IFN-inducible MxA is discussed to be important for control of infections with hantaviruses. To characterize the role of endogenous MxA, the inhibition of HTNV induced by type I and type II IFNs was compared in Vero and A549 cells. IFNα and IFNγ reduced production of infectious virions, viral RNA, and nucleocapsid protein with the same efficiency, although expression of MxA protein was detectable only in IFNα-treated A549 cells. Furthermore, knock down of MxA expression did not impair IFNα-induced inhibition. Thus, inhibition of HTNV induced by type I and type II IFNs did not dependent on expression of endogenous MxA. Taken together, these data suggest that MxA endogenously expressed in response to type I or type II IFNs does not play a pivotal role for the antiviral state against HTNV and that there is more than one mechanism by which cellular defences block hantavirus replication.

Keywords: Antiviral state; MxA; Interferon-alpha; Interferon-gamma; A549; Vero; RNA viruses

1. Introduction

The rodent-borne hantaviruses belong to the family of the *Bunyaviridae* characterized by a tripartite single-stranded RNA genome. The three genomic segments are designated according to their size as S (small), M (medium), and L (large) RNA and code for the nucleocapsid (N) protein, the envelope glycoproteins, and the RNA-dependent RNA polymerase, respectively. Hantaviruses are transmitted to humans by inhalation of aerosols of excreta from infected rodents, in which the virus persists without any pathogenic symptoms. In humans infection can lead to hantavirus cardiopulmonary syndrome (HCPS), hemorrhagic fever with renal syndrome (HFRS), or nephropathia epidemica, a milder variant of HFRS, depending on the species of hantavirus involved. HCPS caused by Sin Nombre or Andes virus have case fatality ratios of approximately 40%. HFRS caused by Hantaan virus (HTNV), Seoul virus, or variants of Dobrava–Belgrade virus is associated with fatality rates between 0.1% and 15% (Kruger et al., 2001; Muranyi et al., 2005; Klempa et al., 2005; Maes et al., 2004). The reasons for the varying degrees of virulence and outcome of a hantavirus infection in humans are still elusive.

Infection with hantaviruses triggers innate immune responses associated with the expression of type I interferon (IFN) alpha/beta (Kraus et al., 2004; Pensiero et al., 1992). Binding of the IFNs to type I IFN-specific receptors on the cell surface activates the protein tyrosine kinases TYK2 and JAK1 and leads to phosphorylation of the cytoplasmic transcription factors STAT1 (signal transducer and activator of transcription) and STAT2 (Brierley and Fish, 2005; Darnell, 1997; Stark et al., 1998). Together with the interferon response factor 9, STAT1 and STAT2 form the trimeric IFN-stimulated gene factor 3, which shuttles into the nucleus and binds to IFN-stimulated response elements located within the promoter region of type I IFN-stimulated genes (ISGs). Jak1 kinase and STAT1 are involved not only in type I but also in type II IFN-triggered signal transduction. Binding of IFNγ to the type II IFN-specific

Abbreviations: IFN, interferon; HTNV, Hantaan virus; N, nucleocapsid protein; HCPS, hantavirus cardiopulmonary syndrome; HFRS, hemorrhagic fever with renal syndrome; NE, nephropathia epidemica; STAT, signal transducer and activator of transcription; ISGs, IFN-stimulated genes; MOI, multiplicity of infection

* Corresponding author. Tel.: +49 30 450525091; fax: +49 30 450525907.
E-mail addresses: r.oelschlegel@gmx.de (R. Oelschlegel), detlev.kruger@charite.de (D.H. Krüger), andreas.rang@charite.de (A. Rang).

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receptor activates the JAK1 and JAK2 kinases which leads to phosphorylation and dimerization of STAT1. The STAT1 homodimer translocates into the nucleus and binds IFNγ activated sequences in promoter regions of certain ISGs (Decker et al., 1997; Platanias, 2005). Type I and type II IFNs induce the expression of a huge pattern of different ISGs, which results in establishment of an antiviral state protecting cells against infection by several viruses, including hantaviruses (Der et al., 1998; Nam et al., 2003; Tamura et al., 1987; Temonen et al., 1995; Wichmann et al., 2002; Staeheli, 1990). Which set of ISGs is expressed determines the efficiency of an antiviral state and depends both on the type of cell or tissue and on the type of IFN involved (Der et al., 1998). From the more than hundred ISGs known, ectopic expression of the human MxA protein was shown to block infection of HTNV in vitro and is been discussed to be important for control of infections with hantaviruses in vivo (Kraus et al., 2004; Khaiboullina et al., 2005; Frese et al., 1996). In the presented study the inhibition of HTNV induced in different cell lines by type I and type II IFN was compared, in order to determine the antiviral state against HTNV in more detail.

2. Materials and methods

2.1. Cell culture, virus, infection, and IFNs

Vero (C1008, clone of VERO 76, LGC Promochem GmbH, Wesel, Germany) and A549 cells (LGC Promochem GmbH) were cultured in Dulbecco's modified Eagle medium supplemented with 5% fetal calf serum, 100 IU penicillin, and 100 μg/ml streptomycin. A stock of the HTNV, strain 76–118 (Lee et al., 1978), was produced in Vero cells after the virus has been focus purified three-times as described recently (Rang et al., 2006). Cells were cultivated in six-well plates and infected with the indicated multiplicity of infection (MOI) in 200 μl for one hour at 37 °C. After infection cells were washed with PBS and fed with fresh medium. Virus stocks and cells were determined to be free of mycoplasma by using the VenorGeM mycoplasma detection kit (Minerva Biolabs, Berlin, Germany). If indicated the cells were treated with IFNα2a (Roferon-A, Roche Deutschland Holding GmbH, Grenzach-Wyhlen, Germany) or IFNγ (Immuno Tools GmbH, Friesoythe, Germany) 1 day before and directly after infection with HTNV.

2.2. Determination of virus titers

The amount of infectious virus secreted into the supernatant of infected cells was quantified as described previously with minor modifications (Heider et al., 2001). Briefly, Vero cells grown in 24-well plates were infected by different dilutions of the harvested supernatants for 1 h at 37 °C, fed with medium containing 0.5% agarose, and incubated for 7 days at 37 °C. Thereafter, the medium/agarose overlay was removed, and cells were washed with PBS and fixed with methanol for 10 min. HTNV-infected cell foci were detected using a polyclonal serum from a rabbit immunized with recombinant N protein from HTNV strain Fojnica (Razanskiene et al., 2004). The formed antigen–antibody complexes were visualized using Chemiluminescence super signal west dura according to the protocol supplied by the manufacturer (Pierce, Perbio, Bonn, Germany). The number of antigen-positive foci was counted to determine the corresponding virus titers which are expressed as focus forming units (FFU) per milliter.

2.3. RNA isolation, Northern blot

Total RNA from Vero and A549 cells was isolated using the TriPure Isolation Reagent (Roche, Mannheim, Germany) according to the manual supplied. The RNA used for RT-PCR analysis was treated with RNase-free DNase I (Roche, Mannheim, Germany) for 30 min at 37 °C to remove genomic DNA which might be present within the samples. Thereafter, the RNA was purified by phenol/chloroform extraction, precipitated, washed twice in 75% ethanol, and stored at −80 °C until further use. For Northern blot analysis 15 μg total RNA was separated on a 1.2% agarose/formaldehyde gel, blotted onto Hybond N nylon membranes (Amersham, International plc, Buckinghamshire, England) and hybridized with ^{32}P-labelled DNA-probes specific for the L-RNA [nucleotide position (ntd-pos.) 5743–6466 according to accession number (Acc-No.) GI 38371716], M-RNA (ntd-pos. 2657–3397; Acc-No. GI 325412), and S-RNA (ntd-pos. 56–1504; Acc-No. NC 005302) of the HTNV genome. Probes were generated using a random-primed labeling kit (Amersham International, Buckinghamshire, England) according to the supplied protocol. Blots were exposed to the Phosphoimager S1 (Molecular Dynamics, Sunnyvale, USA).

2.4. Western blot analysis

Protein extracts were prepared as described previously (Rang et al., 1999). Extracts were separated in a 10% SDS-PAGE and blotted onto nitrocellulose membrane (Whatman GmbH, Dassel, Germany). HTNV nucleocapsid, MxA, and STAT2 protein were detected with N-specific polyclonal sera from a rabbit immunized with recombinant N protein from the HTNV strain Fojnica (Razanskiene et al., 2004), the MxA-specific monoclonal antibody M143 (Flohr et al., 1999), and the STAT-specific monoclonal antibody sc-1668 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) respectively. Signals were visualized by enhanced chemiluminescence (Pierce, Perbio, Bonn, Germany).

2.5. RT-PCR analysis

Complementary DNA (cDNA) was transcribed from 2 μg of total RNA using random hexamer primers and the Moloney Murine Leukemia Virus reverse transcriptase (RT, Invitrogen, Karlsruhe, Germany) for 1 h at 37 °C following the recommendations of the manufacturer. Five percent of the cDNA produced was used as template for PCR. All PCRs contained 1 U Taq DNA Polymerase (Rapidozym, Berlin, Germany) in 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, with 200 μM of each dNTP, and 0.2 μM of forward and reverse primers in a final volume of 50 μl. PCR conditions were 35 cycles of 20 s at 94 °C, 20 s at 55 °C, and 1.5 min at 72 °C, with 4 min at 94 °C prior to the