Journal of Immunological Methods xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Research paper

Development of monoclonal antibodies to Rift Valley Fever Virus and their application in antigen detection and indirect immunofluorescence☆

Claudia Mroz[a], Kristina M. Schmidt[a], Sven Reiche[b], Martin H. Groschup[a], Martin Eiden[a,*]

[a] Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald, Isle of Riems, Germany
[b] Department of Experimental Animal Facilities and Biorisk Management, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald, Insel Riems, Germany

ARTICLE INFO

Keywords:
Rift Valley Fever virus (RVFV)
Monoclonal antibodies
Immunoassays
Kinetics of antigen expression

ABSTRACT

Rift Valley fever virus is a mosquito-borne virus which is associated with acute hemorrhagic fever leading to large outbreaks among ruminants and humans in Africa and the Arabian Peninsula. RVFV circulates between mosquitoes, ruminants, camels and humans, which requires divergent amplification and maintenance strategies that have not been fully explored on the cellular and molecular level. We therefore assessed monoclonal antibodies for their applicability to monitor the expression pattern and kinetics of viral proteins in different RVFV infected cell species. Sequences of RVFV vaccine strain MP-12 were used in a bacterial expression system to produce recombinant non-structural proteins directed to NSs and NSm. After immunization of balb/c mice a set of monoclonal antibodies were generated and extensively characterized. The kinetics of RVFV proteins in vertebrate (Vero76) and mosquito-derived (C6/36) cells were evaluated with monoclonal antibodies against the nucleocapsid protein (NP) and the glycoproteins (Gn and Gc) as well as with the newly generated NSs and NSm derived monoclonal antibodies.

Significant differences of viral protein distribution and accumulation in vertebrate compared to mosquito-derived cells could be demonstrated. Differences were observed for the nonstructural NSm and most intriguingly for the NSs protein indicating significant divergency of replication strategies of RVFV in Vero 76 cells and C6/36 cells. The described monoclonal antibodies are therefore powerful tools to elucidate the discrepancies of virus replication and interaction within the mammalian host compared to the mosquito vector.

1. Introduction

Rift Valley fever (RVF) is an emerging zoonotic disease caused by Rift Valley fever phlebovirus (Bird et al., 2009) out of the novel designated family *Phenuiviridae,* order *Bunyavirales (ICTV, 2017)*. Widely spread throughout many African countries as well as the Arabian Peninsula the corresponding Rift Valley fever virus (RVFV) poses an important public health threat. It occurs in irregular intervals and is characterized by fever, hepatitis, neonatal mortality and abortions in livestock mainly in sheep (Bird et al., 2009; Gerdes, 2004). In humans the disease is often characterized by a self-limited, flu-like illness. Manifestations of the disease, including hemorrhagic fever syndromes, ocular disease or encephalitis can be observed in severe forms in 1–2% of cases (Bird et al., 2009; Ikegami and Makino, 2011; Madani et al., 2003). RVFV can be transmitted by more than 30 mosquito species out of six genera mainly to ruminants and camels, which serve as amplifying vectors (Chevalier et al., 2010). During inter-epidemic periods the virus circulates at low levels in mosquito vectors and is transmitted transovarially by infected *Aedes* floodwater mosquitos (Gerdes, 2002). A transition to an epidemic cycle can be initiated by heavy rainfalls accompanied by a subsequent increase of the infected mosquito population leading to increased virus transmission to susceptible hosts as well as a broad amplification by secondary arthropod vectors like *Culex* or *Anopheles* species (Bird et al., 2009; Gerdes, 2002).

Similarly to all members of the order *Bunyavirales* the RVFV is an enveloped RNA virus with a three segmented genome in negative or ambisense polarity (Bouloy and Weber, 2010). The L segment encodes for the RNA-dependent RNA polymerase L. The M segment contains the genetic information for the glycoproteins Gn and Gc and two accessory proteins, a 14 kDa nonstructural protein (NSm) and a differentially expressed 78 kDa protein (Bird et al., 2009; Bouloy and Weber, 2010; Gerdes, 2004; Pepin et al., 2010). The glycoproteins are highly immunogenic and induce the formation of neutralizing antibodies in infected hosts (Boshra et al., 2011). The nonstructural protein NSm serves as a virulence factor by suppressing the virus-induced apoptosis of host cells (Won et al., 2006). NSm might also play an important role in the

☆ The work was conducted at the Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald - Isle of Riems, Germany

* Corresponding author at: Institute of Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald, Isle of Riems, Germany.
E-mail address: martin.eiden@fli.de (M. Eiden).

https://doi.org/10.1016/j.jim.2018.06.006
Received 9 March 2018; Received in revised form 25 May 2018; Accepted 6 June 2018

mosquito vector, since mutants lacking the NSm protein showed a lower infection rate in mosquitos (Kading et al., 2014). The role of the 78 kDa protein is still not fully understood but it might also play a role during virus replication in mosquitos (Kreher et al., 2014; Weingartl et al., 2014). The S segment utilizes an ambisense strategy. In negative orientation the S segment encodes for the nucleoprotein, which forms ribonucleoproteins associated with the RNA-dependent RNA polymerase L. Another nonstructural protein, the NSs is encoded in antisense orientation (Ikegami and Makino, 2011). As a major virulence factor of RVFV the NSs interferes with the host innate immune response. This includes blocking interferon-ß (IFN-ß) gene expression through different pathways, downregulation of the protein kinase R and by interacting with the host cell transcription factor TFIIH in vertebrates (Billecocq et al., 2004; Ikegami et al., 2009; Le May et al., 2004). As a result, viral replication and proliferation of infected cells are promoted. During infection, NSs forms filamentous structures in the nucleus of infected cells by interacting with regulatory DNA regions leading to chromosome cohesion and segregation defects (Benferhat et al., 2012; Ly and Ikegami, 2016; Mansuroglu et al., 2010). However, in insect cells the function and distribution of these viral proteins are not fully understood. Therefore, the question arose whether these proteins show differences in their expression and localization due to differences in their function during infection in mammalian hosts and mosquito vectors. To investigate this further, we undertook a comparative expression study of RVFV proteins in mammalian Vero 76 and *Aedes*-derived C6/36 cells infected with the attenuated RVFV vaccine strain MP12. Visualizations of the proteins were performed with indirect immunofluorescence assays and immunoblotting utilizing monoclonal antibodies (mabs) against NP, Gn, Gc as well as newly generated monoclonal antibodies against NSs and NSm.

2. Material and methods

2.1. Cells and virus

The SP2/0 myeloma cells, the mammalian Vero 76 cells, and the insect C6/36 cells were obtained from the Collection of Cell Lines in Veterinary Medicine, Friedrich-Loeffler-Institute, Germany. The SP2/0 myeloma cells were grown in RPMI 1640 media (Gibco, life technologies, Thermo fisher, Germany) at 37 °C in vented flasks. The Vero and C6/36 cell lines were grown in minimal essential media with 10% fetal bovine serum (FBS). Vero cells were incubated at 37 °C and C6/36 cells at 28 °C in closed flasks. The RVFV MP12 vaccine strain was kindly supplied by Richard Elliot (University of Glasgow, Center for virus research) and was handled under BSL-2 conditions. Virus titer was determined as 50% tissue culture infective doses ($TCID_{50}$) using Vero 76 which yielded a titer of $10^{6,9}$ $TCID_{50}$/ml. Calculation was carried out by the Spearman-Kärber method (Kärber, 1931; Spearman, 1908).

2.2. Expression of recombinant NSs and NSm protein

RNA was isolated from cell culture supernatant from RVFV MP12-infected cells using a QIAamp Viral RNA Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. The genes encoding the nonstructural protein NSs and NSm of RVFV MP12 were amplified by a one-step RT-PCR. The amplification of the gene encoding the NSs protein was performed from the S segment with NSS_3 (5′-CCGGATC CGATTACTTTCCTGTGATATCTG-3′) as forward primer and NSs_4 (5′-CCAAGCTTCTAATCAACCTCAACAAATC-3′) as reverse primer. These primers contain a *BamH1* and a *HindIII* restriction site, respectively. The gene encoding the NSm protein was amplified from the M segment using NSm_4 (5′ CCGAATTCATTATTAGAGTGTCTCTAAGCTCC-3′) as forward primer and NSm_5 (5´-CCCTCGAGAGCAAAAACAACAGGTGC CAAAGC-3′) as reverse primer. These primers contain an *EcoRI* and *XhoI* restriction site, respectively. All primers were synthesized and HPLC purified by Eurofins MWG Operon (Ebersberg, Germany).

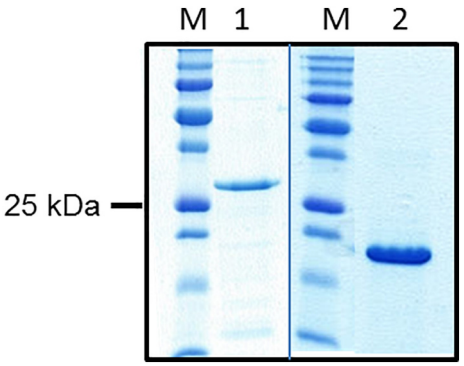

Fig. 1. SDS Page analysis and Coomassie blue staining of recombinant NSs and NSm protein. (M) Molecular weight marker. (1) recombinant NSs protein (2) recombinant NSm protein. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Amplification by one-step RT-PCR was performed using a Super Script III One-Step RT-PCR with Platinum Taq Kit (Invitrogen, USA) according to the manufacturer's instructions. The amplified NSs and NSm genes were sub-cloned into pBluescriptK/S vector (Agilent Technologies, Denmark) and amplified in XL1-blue *Escherichia coli* cells (Invitrogen). The NSs gene was subsequently cloned into the bacterial expression vector pQE40 (Qiagen) via the restriction sides *BamH1* and *HindIII* (New England Bio Labs, Germany). The restriction sides *XhoI* and *EcoR1* (New England Bio Labs) were used to clone the NSm gene into the bacterial expression vector pET21a (Novagen, UK). The corresponding clones were designed as Nss_pQE40 and Nsm_pET21a, respectively. Expression and purification of recombinant RVFV NSs and NSm proteins were carried out under denaturing conditions as described previously by Jäckel (Jackel et al., 2013).

2.3. Immunization of BALB/c mice and hybridoma cell preparation

Four BALB/c mice per antigen were immunized intraperitoneally with 100 µg of recombinant NSs or NSm protein on days 0, 30, 60 and 90. A final boost with 100 µg protein solution was carried out three days before spleen cells were fused with SP2/0 myeloma cells in a ratio of 1:4 in the presence of polyethylene glycol 1500 (PEG, Sigma-Aldrich). Hybridoma cells were selected in RPMI 1640 media (Gibco, life technologies, Thermo fisher, Germany) with hypoxanthine-aminopterin-thymidine (HAT) selective medium (Sigma-Aldrich) and 10% foetal calf serum (life technologies), BM Condimed H1 (Hybridoma cloning supplement, Sigma-Aldrich) non-essential amino acids (life technologies), L-glutamine (200 mM, life technologies), penicillin (10,000 units, life technologies), streptomycin (10 mg/ml, life technologies), and sodium pyruvate (100 mM, life technologies). Stepwise the HAT medium was replaced by hypoxanthine-thymidine (HT) medium (Sigma-Aldrich) followed by maintenance media. Supernatants of hybridoma clones were screened for anti-NSs and anti-NSm antibodies by indirect ELISA followed by characterization by western blotting and indirect immunofluorescence. The antibody classes were determined using the commercial Pierce Rapid ELISA Mouse mAb Isotyping Kit (Thermo Fisher) according to the manufacturer's instructions. Experiments were performed in compliance with national and European legislation, and were approved by the competent authority of the Federal State of Mecklenburg-Western Pomerania, Germany.

2.4. Enzyme-linked immunosorbent assay (ELISA)

Mouse polyclonal serum and hybridoma supernatants were assessed with an indirect ELISA previously published by Jäckel (Jackel et al., 2014). In short, Maxisorb immunoplates (Nunc, Denmark) were coated overnight at 4 °C with 1 µg/ml recombinant NSs or NSm protein. After