Virology 515 (2018) 250–260

Contents lists available at ScienceDirect

Virology

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

Ross River virus envelope glycans contribute to disease through activation of the host complement system

Bronwyn M. Gunn[a,1], Jennifer E. Jones[a], Reed S. Shabman[a,2], Alan C. Whitmore[b], Sanjay Sarkar[b], Lance K. Blevins[b,3], Thomas E. Morrison[c], Mark T. Heise[a,b,*]

[a] Dept. of Microbiology and Immunology, University of North Carolina at Chapel Hill, 160 Dental Circle, 9024 Burnett Womack, Chapel Hill, NC 27599, USA
[b] Dept. of Genetics, University of North Carolina at Chapel Hill, 160 Dental Circle, 9024 Burnett Womack, Chapel Hill, NC 27599, USA
[c] Dept. of Microbiology, University of Colorado School of Medicine, 12800 E. 19th Ave., RC1N 9119, Mail Stop 8333, Aurora, CO 80045, USA

ARTICLE INFO

Keywords:
Ross River virus
Alphavirus
Mannose-binding lectin
Complement
N-linked glycan

ABSTRACT

Mannose binding lectin (MBL) generally plays a protective role during viral infection, yet MBL-mediated complement activation promotes Ross River virus (RRV)-induced inflammatory tissue destruction, contributing to arthritis and myositis. As MBL binds to carbohydrates, we hypothesized that N-linked glycans on the RRV envelope glycoproteins act as ligands for MBL. Using a panel of RRV mutants lacking the envelope N-linked glycans, we found that MBL deposition onto infected cells was dependent on the E2 glycans. Moreover, the glycan-deficient viruses exhibited reduced disease and tissue damage in a mouse model of RRV-induced myositis compared to wild-type RRV, despite similar viral load and inflammatory infiltrates within the skeletal muscle. Instead, the reduced disease induced by glycan-deficient viruses was linked to decreased MBL deposition and complement activation within inflamed tissues. These results demonstrate that the viral N-linked glycans promote MBL deposition and complement activation onto RRV-infected cells, contributing to the development of RRV-induced myositis.

1. Importance

Mannose binding lectin (MBL), a lectin that can initiate the host complement cascade, generally has a protective role following viral infection. However, in the context of alphavirus-induced disease, MBL-mediated complement activation is pathologic, promoting Ross River virus (RRV)-induced inflammatory tissue destruction within the musculoskeletal system. MBL binds to glycosylated proteins, thus we hypothesized that the RRV envelope N-linked glycans promote MBL binding, complement activation, and disease. Using a panel of mutant viruses lacking one or more envelope glycans, we found that the RRV E2 glycans are required for MBL binding to infected cells and subsequent disease. Mice infected with a virus lacking both E2 glycans exhibited reduced disease and tissue damage, and decreased MBL binding and complement activation compared to mice infected with the wild type virus. These results suggest that interactions between MBL and the viral N-linked glycans play a major role in development of alphavirus-induced inflammatory disease.

2. Introduction

Arthritogenic alphaviruses such as Ross River virus (RRV) and chikungunya virus (CHIKV) are mosquito-borne viruses that cause outbreaks of infectious arthritis and myositis in many regions of the world. Both RRV and CHIKV share similar disease symptoms that are characterized by debilitating polyarthritis and myositis that frequently results in myalgia and arthralgia. Studies in both humans and mice have identified a critical role for the host inflammatory response in the development of disease and immunopathology following infection, with macrophages playing an essential role in damage to the musculoskeletal system (Lidbury et al., 2000; Morrison et al., 2006; Herrero et al., 2013, 2011).

We have previously demonstrated that the host complement system initiated by mannose binding lectin (MBL) also has a critical role in development of disease (Gunn et al., 2012). Interestingly, rather than regulating the infiltration of macrophages and other inflammatory cells into the musculoskeletal system, MBL-mediated complement activation

* Corresponding author.
E-mail addresses: bgunn@mgh.harvard.edu (B.M. Gunn), jejxyz@email.unc.edu (J.E. Jones), alan_whitmore@med.unc.edu (A.C. Whitmore), sanjay_sarkar@med.unc.edu (S. Sarkar), thomas.morrison@ucdenver.edu (T.E. Morrison), mark_heisem@med.unc.edu (M.T. Heise).
[1] Present address: Ragon Institute of MGH, MIT, and Harvard, Cambridge, MA 02139, USA.
[2] Present address: ATCC, Gaithersburg, MD, USA.
[3] Present address: Center for Integrative Toxicology, Michigan State University, USA.

https://doi.org/10.1016/j.virol.2017.12.022
Received 20 July 2017; Received in revised form 24 November 2017; Accepted 20 December 2017
Available online 08 January 2018
0042-6822/

following RRV infection contributes to disease by regulating inflammatory cell activation within the inflamed tissue through complement receptor 3 (CR3) (Gunn et al., 2012; Morrison et al., 2008). The C3 cleavage product iC3b produced following complement activation can bind to CR3 on inflammatory cells including macrophages and neutrophils to induce phagocytosis of iC3b-opsonized pathogens, and can also initiate cytotoxic effector programs through Syk-mediated outside-in signaling that leads to tissue damage in autoimmune disorders (Ross, 2000; Abram and Lowell, 2009; Hirahashi et al., 2006). In the absence of MBL, C3, or CR3 expression of cytotoxic inflammatory mediators within muscle tissue are reduced in RRV-infected mice, correlating with reduced disease, but viral tropism and viral burden are unaffected (Gunn et al., 2012; Morrison et al., 2008). Importantly, production of the CR3 ligand iC3b is reduced in the absence of MBL following RRV infection (Gunn et al., 2012), suggesting that MBL-mediated complement activation regulates production of iC3b and subsequent activation phenotype of CR3-bearing inflammatory cells present within the tissues.

MBL-mediated complement activation is initiated by the recognition of terminal sugars on glycosylated proteins by the carbohydrate recognition domain (CRD) of MBL (reviewed in Takahashi et al., 2006). The alphavirus glycoproteins E1 and E2 contain three to four N-linked glycosylation sites that are glycosylated with N-linked glycans (Strauss and Strauss, 1994). During structural protein synthesis, the E2 and E1 glycoproteins are processed through the ER and Golgi to generate mature E2-E1 heterodimers, which self-assemble into trimeric spikes at the plasma membrane. Alphaviruses bud from the plasma membrane and each budding alphavirus virion incorporates 80 glycoprotein spikes to make up the viral envelope. The E2 glycoprotein is prominently displayed on the surface of the virus and on the surface of infected cells, and for most alphaviruses, two of the N-linked glycans are located on E2. The RRV E2 N200 glycan is located on one side of the tip of the protruding E2 “petal” on the glycoprotein spike (E2 N200), and the E2 N262 glycan appears to be located between the trimeric spikes (Pletnev et al., 2001). Thus, the RRV E2 N-linked glycans are surface exposed and are in a key position to interact with host proteins. The RRV E2 N-linked glycans are glycosylated with a combination of high mannose (E2 N200) and complex (E2 N262) glycans when produced in mammalian cells (Shabman et al., 2008). Therefore, while we have previously demonstrated that MBL does not directly bind to free RRV virions (Gunn et al., 2012), we hypothesized that MBL might interact with the N-linked glycans on the RRV envelope glycoproteins when they are displayed on the surface of infected cells.

In this study, we used RRV mutants that lack one or both of the two N-linked glycosylation sites on E2 to demonstrate that the E2 N-linked glycans are required for MBL binding to infected cells and subsequent induction of virus-induced disease. While RRV-infected cells are readily bound by MBL, this activity was lost when the cells were infected with RRV lacking both N-linked glycans on the viral E2 glycoprotein. Furthermore, viruses lacking either E2 glycosylation site caused reduced RRV disease in mice, and a virus lacking both sites was further attenuated. Our results support a model of RRV pathogenesis wherein the E2 N-linked glycans promote activation of the lectin complement pathway by MBL, resulting in activation of CR3-bearing inflammatory cells and subsequent damage within inflamed tissues.

3. Materials and methods

3.1. Ethics statement

All mouse studies were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All mouse studies were performed at the University of North Carolina (Animal Welfare Assurance #A3410-01) using protocols approved by the University of North Carolina Institutional Animal Care and Use Committee (IACUC; Protocol #10–204 and 11–224). All studies were performed in a manner designed to minimize pain and suffering in infected animals, and any animal that exhibited severe disease signs was euthanized immediately in accordance with IACUC approved endpoints.

3.2. Viruses and cells

WT RRV is derived from the infectious clone of RRV T48 (pRR64), and the E2 N-linked glycan mutants were generated previously by site directed mutagenesis of N-linked glycosylation sites in E2 in pRR64 (Shabman et al., 2008). The viral stocks used in this study were generated as described in (Morrison et al., 2006). Primary myoblasts were generated from skeletal muscle from one day-old C57BL/6J mice, and muscle was dissociated by type I collagenase (Worthington Biochemicals) and grown in DMEM supplemented with 6% FBS. To differentiate cells, the medium was replaced with DMEM containing 3% FBS. Baby hamster kidney (BHK-21) cells were cultured in MEM alpha supplemented with 10% FBS and L-glutamine. Human embryonic kidney (HEK) 293T cells were cultured in DMEM supplemented with 10% FBS.

3.3. MBL deposition onto myotubes

Differentiated myotubes (C2C12 or primary cells from C57BL/6J mice) were either mock-infected or infected with RRV WT or E2 DM at an MOI of 20. At 18 hpi, culture medium was removed and cells were incubated in medium containing either 10% serum from WT or MBL-DKO mice for an additional 30 min. Cells were washed with PBS containing 400 mM NaCl and harvested in lysis buffer. Cell lysates were analyzed by immunoblot analysis by standard techniques. Densitometry was performed using ImageJ (NIH), and values were normalized either to actin, RRV E2, or RRV capsid.

3.4. Immunofluorescence

BHK-21 cells were infected at MOI of 1 with either diluent alone (mock), RRV WT or E2 DM. At 12 hpi, medium was removed and cells were incubated in HBSS with 5 mM $CaCl_2$ with or without 10 μg/ml rhMBL (R&D Systems) for 30 min. Cells were washed with PBS, fixed with PFA, and stained using standard techniques using the following antibodies: α-MBL-C (SCBT 1:50); α-RRV (ATCC 1:1000); Alexa Fluor 488-α mouse (Invitrogen 1:1000); and Alexa Fluor 594 α-rabbit (Invitrogen 1:1000). Slides were mounted with ProLong Gold with DAPI, (Invitrogen) and imaged by fluorescence microscopy. Images were processed using ImageJ (NIH).

3.5. RRV antigen detection by flow cytometry

BHK-21 or HEK 293T cells were infected at an MOI of 5 with either diluent (mock), RRV WT, or the indicated RRV E2 mutants. At 12 hpi, media was removed, cells were washed with PBS, and cells were harvested using cell dissociation buffer (Gibco). Cells were then spun down and stained for extracellular RRV antigen using α-RRV (ATCC) and FITC-conjugated α-mouse (eBioscience). Cells were then fixed in 4% PFA and analyzed by flow cytometry as previously described (Morrison et al., 2008).

3.6. Mice

All mice used in this study were maintained and bred in house at the University of North Carolina (UNC) in accordance with UNC Institutional Animal Care and Use Committee guidelines. C57BL/6J mice were purchased from The Jackson Laboratories (Bar Harbor, ME).

3.7. Infection of mice with RRV

24-day-old C57BL/6J mice were inoculated with 10^3 PFU of RRV in