Antiviral Research 146 (2017) 112–120

Contents lists available at ScienceDirect

Antiviral Research

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

# Identification of broadly neutralizing monoclonal antibodies against Crimean-Congo hemorrhagic fever virus

Marko Zivcec, Lisa I.W. Guerrero, César G. Albariño, Éric Bergeron, Stuart T. Nichol, Christina F. Spiropoulou*

*Viral Special Pathogens Branch, Division of High Consequence Pathogens and Pathology, Centers for Disease Control and Prevention, Atlanta, GA, USA*

ARTICLE INFO

*Article history:*
Received 26 June 2017
Received in revised form
17 August 2017
Accepted 22 August 2017
Available online 24 August 2017

*Keywords:*
Crimean-Congo hemorrhagic fever virus
Virus-like particles
Monoclonal antibodies
Neutralization assay

ABSTRACT

Despite the serious public health impact of Crimean-Congo hemorrhagic fever (CCHF), the efficacy of antivirals targeting the causative agent, CCHF virus (CCHFV), remains debatable. Neutralizing monoclonal antibodies (MAbs) targeting the CCHFV glycoprotein Gc have been reported to protect mice against challenge with the prototype CCHFV strain, IbAr10200. However, due to extensive sequence diversity of CCHFV glycoproteins, it is unknown whether these MAbs neutralize other CCHFV strains. We initially used a CCHF virus-like particle (VLP) system to generate 11 VLP moieties, each possessing a glycoprotein from a genetically diverse CCHFV strain isolated in either Africa, Asia, the Middle East, or southeastern Europe. We used these VLPs in biosafety level 2 conditions to efficiently screen MAb cross-neutralization potency. Of the 16 MAbs tested, 3 (8A1, 11E7, and 30F7) demonstrated cross-neutralization activity with most CCHF VLPs, with 8A1 neutralizing all VLPs tested. Although binding studies suggest that none of the MAbs compete for the same epitope, combining 11E7, 30F7, or both 11E7 and 30F7 with 8A1 had no additive effect on increasing neutralization in this system. To confirm our findings from the VLP system, the 3 MAbs capable of strain cross-neutralization were confirmed to effectively neutralize 5 diverse CCHFV strains in vitro. Passaging CCHFV strains in the presence of sub-neutralizing concentrations of MAbs did not generate escape mutants resistant to subsequent neutralization. This study demonstrates the utility of the VLP system for screening neutralizing MAbs against multiple CCHFV strains, and provides the first evidence that a single MAb can effectively neutralize a number of diverse CCHFV strains in vitro, which may lead to development of future CCHF therapeutics.

Published by Elsevier B.V.

## 1. Introduction

Crimean-Congo hemorrhagic fever virus (CCHFV) is the causative agent of an acute severe viral hemorrhagic fever (CCHF) in humans. Geographically, CCHFV or CCHFV reactive antibodies have been detected throughout Southern Eurasia and Africa (Bente et al., 2013). Case fatality rates of CCHF vary from ~5% to 30% (Bente et al., 2013; Ergonul, 2012; Papa et al., 2016). Due to the broad and expanding geographic distribution of *Hyalomma* ticks, the primary vector and reservoir of CCHFV, CCHF outbreaks may increase in frequency and spread to new areas (Estrada-Peña et al., 2015). Currently, prophylactic and therapeutic options available for treating CCHF patients are limited to administration of the antiviral drug ribavirin, which has not shown clear clinical benefit in historic meta analyses (Duygu et al., 2012; Koksal et al., 2010; Soares-Weiser et al., 2010). Therefore, development of novel CCHFV therapeutics is of utmost importance in combating the increasing public health burden of CCHF.

In recent years, several groups have shown that polyclonal or monoclonal antibodies (MAbs), or combinations of MAbs, can prevent fatal disease when administered to animals experimentally infected with hemorrhagic fever viruses (Cross et al., 2016; Qiu et al., 2014; Zeitlin et al., 2016). Antibody therapy has been attempted in several instances of human CCHF (Kubar et al., 2011; Van Eeden et al., 1985) and has shown modest success in small studies, but its efficacy has not been assessed in large or randomized clinical trials. Furthermore, mouse studies have suggested an important role for antibodies in protection from CCHF (Canakoglu et al., 2015; Dowall et al., 2016). Together, these data suggest that antibody treatment may be an effective therapy against CCHFV, but a number of questions remain.

* Corresponding author.
*E-mail address:* ccs8@cdc.gov (C.F. Spiropoulou).

http://dx.doi.org/10.1016/j.antiviral.2017.08.014
0166-3542/Published by Elsevier B.V.

Mouse studies have shown that immune responses against the CCHFV glycoproteins expressed as the complete glycoprotein precursor, GPC, are important for protection (Buttigieg et al., 2014; Hinkula et al., 2017), while immune responses against partial GPC subunits delay the time to death (Kortekaas et al., 2015). However, the GPC gene is more genetically variable than the other viral proteins such as the nucleocapsid protein (NP) and the viral RNA-dependent RNA-polymerase (L); the encoded surface glycoproteins may vary by over 25% at the amino acid level among strains co-circulating in the same territory (Goedhals et al., 2014; Papa et al., 2014). Thus, GPC may be a more difficult target for the immune system or antibody therapy, especially when heterologous strains of the virus are in concurrent circulation (Flyak et al., 2016; Wec et al., 2016).

Here, we studied the cross-strain neutralization potential of a panel of previously reported MAbs raised against the IbAr10200 prototype CCHFV strain (Bertolotti-Ciarlet et al., 2005). Potent virus neutralization activity has been reported as essential for several successful antibody therapies (Geisbert et al., 2014; Qiu et al., 2014; Wu et al., 2008). We assessed here under biosafety level 2 (BSL-2) conditions the ability of MAbs to cross-neutralize CCHFV strains using the transcription and entry-competent virus-like particle (tecVLP) system. tecVLPs consist of viral proteins and a minigenome that together generate particles that are morphologically consistent with CCHFV. Therefore, they mimic the viral replication cycle and subsequent cell entry and transcription without generating infectious virus. We then confirmed our findings with tecVLPs using CCHFV strains under BSL-4 conditions.

## 2. Materials and methods

### 2.1. Biosafety statement

Procedures involving infectious CCHFV were conducted in a BSL-4 facility according to institutionally approved standard operating procedures. Other procedures were performed under BSL-2 conditions.

### 2.2. Cell lines and antibodies

BSR-T7 cells were obtained from K.K. Conzelmann (Ludwig-Maximilians-Universität) and propagated in DMEM supplemented with 10% FBS, media additives (1% sodium pyruvate, 1% non-essential amino acids, and 1% penicillin/streptomycin), and 400 ng/mL gentamycin. SW-13 cells were obtained from P. Leyssen (Rega Instituut KU) and propagated in DMEM supplemented with 10% FBS and media additives. All cells were grown in a humidified 37 °C, 5% $CO_2$ incubator.

Murine MAbs targeting CCHFV strain IbAr10200 PreGn (5A5, 6B12, 7F5, 8F10, 10E11, 11F6, and 13G8), PreGc (1H6, 3E3, 11E7, 12A9, 13G5, and 30F7), and NP (2B11 and 9D5) were obtained from the Joel M. Dalrymple-Clarence J. Peters USAMRIID Antibody Collection through BEI Resources. Antibodies were purified by protein G affinity chromatography to >95% purity (purity range 97.5–99.8%) as determined by Experion Pro260 analysis. The PreGc MAb 8A1 was produced from hybridoma cells (from G. Ludwig, USAMRIID) by GenScript Inc. 8A1 was purified by protein A affinity chromatography, and had 95% purity as determined by SDS-PAGE. CCHFV hyperimmune mouse ascetic fluid (HMAF) used was the same as previously reported (Bergeron et al., 2015).

### 2.3. Viruses and tecVLPs

The ORFs of the GPC of CCHFV isolates ArD15786 (DQ211627), Baghdad-12 (AJ538197), Kosova Hoti (EU037902), NIV112143 (JN572085), SPU18/88 (KJ682810), Sudan Al-Fulah 3-2008 (HQ378185), and YL04057 (FJ562094) were codon-optimized and synthesized by GenScript Inc, and cloned into the previously described expression vector pCAGGS (pC-GPC). Including the previously described IbAr10200, Oman199809166, Turkey200406546, and Afg09 tecVLPs, 11 tecVLP moieties were generated (Fig. 1) (Zivcec et al., 2015).

The previously described CCHFV helper plasmids encoding the strain IbAr10200 NP (pC-NP), the codon-optimized L (pCLCK-L, possessing an R substitution at position 16) helper plasmids, and the pL-Luc minigenome plasmids were used in all experiments. BSR-T7 cells were transfected with pC-NP, pCLCK-L, pL-Luc, and a pC-GPC plasmid using TransIT-LT1 Transfection Reagent according to manufacturer's recommendations (Mirus Bio LLC). Media were replaced with fresh culture medium the following day, and tecVLP-containing cell supernatants were collected and concentrated as previously described (Zivcec et al., 2015).

CCHFV isolates Turkey-812955 (KY362515, KY362517, and KY362519), Oman-812956 (KY362514, KY362516, and KY362518), UAE-813040 (MF289414, MF289415, and MF289416), and UAE-813042 (MF289417, MF289418, and MF289419) were propagated in SW-13 cells. CCHFV strain IbAr10200 was generated by reverse genetic approaches as previously described, and propagated in SW-13 cells (recIbAr10200) (Bergeron et al., 2015). CCHFV identity and sequence, and exclusion of contaminants in the virus stocks, were confirmed by next-generation sequencing using a MiniSeq System according to manufacturer's instructions (Illumina, Inc).

### 2.4. CCHFV and tecVLP neutralization assay

tecVLP neutralization assays were conducted as previously described (Zivcec et al., 2015). For the CCHFV plaque reduction/neutralization test (PRNT), MAbs were diluted in SW-13 media to equal starting concentrations (10 μg/mL), and further diluted in a 2-fold dilution series (concentration range $10^1$ to $8 \times 10^{-2}$ μg/mL, or ~1:100- to 1:12800-fold dilutions). MAb dilutions were mixed with equal volumes of CCHFV isolates diluted to ~100 $TCID_{50}$/well in SW-13 media, and incubated for 1 h at 37 °C. The mixture was then applied to confluent monolayers of SW-13 cells in 6-well plates and incubated for 1 h at 37 °C. Following incubation, the inocula were removed, and the cells were washed with DMEM, overlaid with 1–1.2% Avicel (FMC Health and Nutrition), and incubated at 37 °C for 3–4 days. The overlays were removed, fixed with formalin, and stained with crystal violet. Plaques were counted visually, and reductions in the number of plaques were reported as the titer reduction percentage.

### 2.5. Serial passaging of CCHFV in the presence of neutralizing MAb

CCHFV strains recIbAr10200 and Turkey-812955 were passaged in the presence of sub-neutralizing doses of 8A1 (0.625 or 0.3125 μg/mL) or 30F7 (1.25 or 0.625 μg/mL), or in SW-13 media. Both strains were incubated with MAbs and used to infect SW-13 cells in 6-well plates as described in 2.4. Following infection, the inocula were replaced with their respective MAb-containing or plain SW-13 media and incubated for 2 days at 37 °C. Following incubation, supernatants were aliquoted and frozen at −80 °C. CCHFV RNA levels were monitored by quantitative RT-PCR assays as previously described (Bergeron et al., 2015; Spengler et al., 2017). Five CCHFV passages were performed; efforts to passage the virus for longer than 5 passages in the presence of sub-neutralizing level of MAbs all resulted in reduction of CCHFV RNA to undetectable levels. Changes in GPC sequences were determined by next-generation sequencing. Briefly, the GPC region of Turkey-812955 and recIbAr10200 was amplified from the extracted RNA using