

## SUPPLEMENT ARTICLE

# Development and Characterization of Broadly Cross-reactive Monoclonal Antibodies Against All Known *Ebolavirus* Species

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**As of 25 March 2015, the largest recorded outbreak of Ebola virus infection is ongoing, with almost 25 000 cases and >10 000 deaths. There are 5 genetically and antigenically distinct species within the genus *Ebolavirus*. Limited cross-reactivity and protection is observed between these 5 *Ebolavirus* species, which complicates vaccine development. However, on the basis of sequence homology between the 5 *Ebolavirus* species, we hypothesize that conserved epitopes are present on the viral glycoprotein (GP), which can be targeted by antibodies. In the current study, a panel of mouse monoclonal antibodies was isolated and characterized using an enzyme-linked immunosorbent assay (ELISA) to determine cross-reactivity, avidity, and competition for epitope binding; Western blot analysis was also performed. Four monoclonal antibodies were identified by ELISA as cross-reacting with the GPs of all 5 *Ebolavirus* species. The identification of cross-reactive antibodies that bind the GPs of all known *Ebolavirus* species will give us important insight into the presence of conserved epitopes on the viral GP. These data will be crucial for the development of novel therapeutics and diagnostic assays.**

**Keywords.** *Ebolavirus*; cross-reactive; antibody.

Ebola virus (EBOV) is an emerging pathogen belonging to the genus *Ebolavirus* in the family *Filoviridae* and is the causative agent of Ebola virus disease [1]. As of 25 March 2015, the largest recorded outbreak of EBOV infection is ongoing, with 24 907 cases and 10 326 deaths [2].

There are 5 genetically and antigenically distinct species of *Ebolavirus*: Bundibugyo virus (BDBV), Tai Forest virus (TAFV), Reston virus (RESTV), Sudan virus (SUDV), and EBOV [1]. Limited cross-reactivity and cross-protection are observed among these 5 *Ebolavirus* species, which complicates development of vaccines

and diagnostic tests. The *Ebolavirus* glycoprotein (GP) mediates viral attachment and entry into host cells and is a major target for the host immune response [3]. Several candidate vaccines are in development, most of which use the GP as the immunogen [4]. Vaccination with GP results in a robust antibody response, which has recently been shown to be a mechanism of protection in nonhuman primates [5, 6]. The development and characterization of monoclonal antibodies (mAbs) has been instrumental in identifying neutralizing epitopes [7, 8]; however, most neutralizing antibodies are *Ebolavirus*-species specific and do not cross-react. Therefore, a mix of several mAbs are needed for broad protection against infection and disease.

We previously identified a mAb (S9) that binds the EBOV GP and neutralizes EBOV but not the other 4 *Ebolavirus* species in vitro. In addition, this mAb protects in vivo against EBOV infection [9]. However, based on the sequence homology among the 5 *Ebolavirus* species, we hypothesized that conserved epitopes are present on the *Ebolavirus* GPs, which can be targeted by antibodies.

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In the current study, we characterized a panel of mAbs that recognize the GP of all known *Ebolavirus* species.

## METHODS

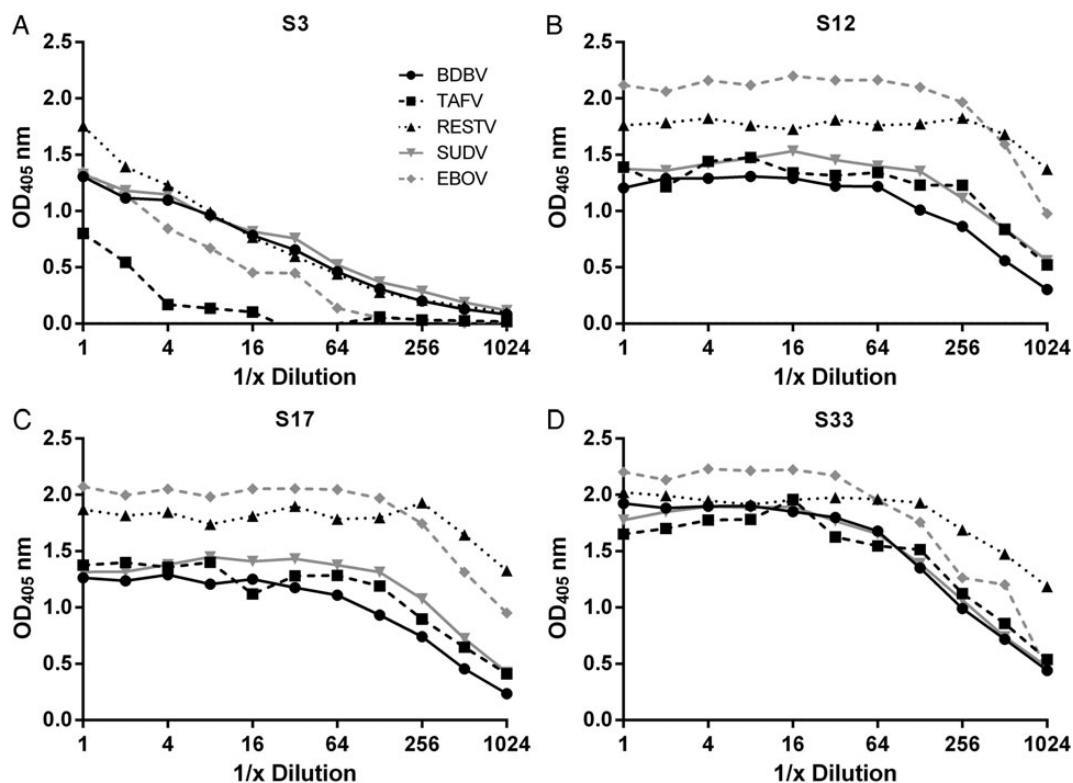
mAbs were previously generated by vaccinating mice with recombinant vesicular stomatitis virus (rVSV) expressing the EBOV GP (rVSV-EBOV-GP) and inducing a cross-reactive memory response by boosting with the heterologous rVSV-SUDV-GP as previously described [9]. rVSV was kindly provided by Dr Andrea Marzi. Briefly, 3 days after boost, hybridoma cells were generated by fusing mouse plasma cells with SP2/0-Ag14 myeloma cells (ATCC) and were selected using HAT/HT selection medium. Monoclonal hybridoma cells were isolated by performing 2 rounds of limiting dilutions in 96-well flat-bottomed tissue culture plates. Isolated hybridoma cells were initially screened by enzyme-linked immunosorbent assay (ELISA) for detection of secreted antibodies, using a soluble, transmembrane-deleted, trimeric glycoprotein of EBOV, SUDV, RESTV, TAFV, and BDBV as previously described [9, 10]. Plasmids encoding for soluble, transmembrane-deleted GP were kindly provided by Dr Ayato Takada.

Approval for animal experiments was obtained from the institutional animal care and use committees at the Rocky

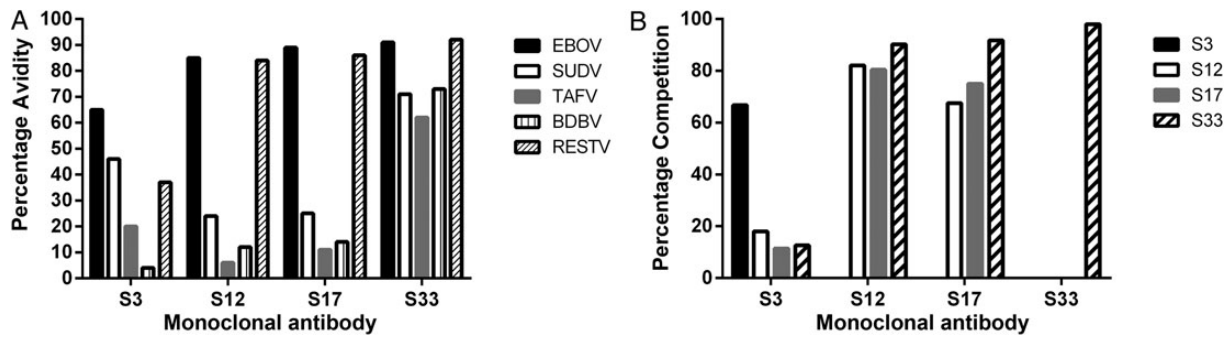
Mountain Laboratories, Division of Intramural Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), and the University of Texas Medical Branch. Animal work was performed by certified staff in an Association for Assessment and Accreditation of Laboratory Animal Care–approved facility. Animal housing, care, and experimental protocols were in accordance with NIH guidelines.

## RESULTS AND DISCUSSION

A total of 33 monoclonal hybridoma cells produced antibodies that bound the GP of  $\geq 1$  *Ebolavirus* species (data not shown). All mAbs that bound EBOV GP were subsequently tested in a plaque reduction assay as previously described [9]. With the exception of the previously published S9 mAb, none of the other mAbs neutralized any of the *Ebolavirus* species (data not shown). In the absence of neutralizing activity, the focus was shifted to cross-reactive mAbs that can bind to all known *Ebolavirus* species. ELISA identified 4 immunoglobulin G1 mAbs (S3, S12, S17, and S33) that bound the GPs of all 5 *Ebolavirus* species, although the reactivity of S3 was typically lower than that of S12, S17, and S33 (Figure 1A–D). This suggested that  $\geq 1$  conserved epitope on the GP was targeted by these mAbs.



**Figure 1.** Cross-reactivity of monoclonal antibodies (mAbs) against 5 *Ebolavirus* species. The reactivity of the 4 mAbs S3 (A), S12 (B), S17 (C), and S33 (D) was determined by an enzyme-linked immunosorbent assay, using the soluble glycoprotein of Bundibugyo virus (BDBV), Tai Forest virus (TAFV), Reston virus (RESTV), Sudan virus (SUDV) and Ebola virus (EBOV) as antigens.



**Figure 2.** The avidity (A) and competition (B) characteristics of 4 monoclonal antibodies was determined by an avidity or competitive enzyme-linked immunosorbent assay, using the soluble glycoprotein of Bundibugyo virus (BDBV), Tai Forest virus (TAFV), Reston virus (RESTV), Sudan virus (SUDV), and Ebola virus (EBOV) as antigens. Data are from 1 representative experiment of 3.

To determine whether linear or conformational epitopes were targeted, mAbs underwent Western blotting to detect the GP of the 5 different *Ebolavirus* species, as previously described [9]. Surprisingly, while ELISA found that all mAbs cross-reacted, Western blotting revealed that only S33 and S17 detected the GP1 from the 5 different species and that S3 and S12 only bound the EBOV GP (data not shown). Detection of the EBOV GP suggests that the mAbs recognized  $\geq 1$  linear epitopes on the GP1 but that these epitopes are only partially conserved, since the same epitope on the GP of other *Ebolavirus* species was not always detected.

To determine whether the difference in Western blot findings among the *Ebolavirus* species could be due to differences in the avidity of the mAbs for the GP epitopes of the 5 species, we adapted the ELISA. Briefly, a 5-minute incubation step with or without 8 M urea was added after aspiration of the mAbs, and the avidity index was calculated as previously described for noroviruses, as follows:  $[(\text{OD with urea})/(\text{OD without urea})] \times 100$  [11]. The avidity for all mAbs was highest for the EBOV GP used during primary vaccination (Figure 2A). The avidity of mAb S33 was higher for all GPs, compared with avidities of the other mAbs. Interestingly, mAbs S12 and S17 showed the highest avidity for RESTV, which was not part of the immunization scheme. Surprisingly, while SUDV was used as a vaccination boost, most mAbs, except mAb S33, did not have high avidity for this antigen.

To determine whether these 4 mAbs recognized the same epitope, we determined inhibition of the binding of biotinylated mAbs S3, S12, S17, and S33 to the EBOV GP by using the same panel of unlabeled mAbs (at saturated concentrations). The percentage of binding competition was calculated as  $[1 - (\text{OD competition})/(\text{OD maximum binding of biotinylated mAbs})] \times 100$ , as previously described for mAbs against SARS-CoV [12]. mAbs S12, S17, and S33 did not compete with S3; therefore, S3 recognized a unique epitope on the GP (Figure 2B). mAbs S12, S17, and S33 competed with both mAbs S12 and S17 with similar efficacy. Surprisingly, although mAb S33 competed

with mAbs S12 and S17 in this assay, reciprocal competition was not observed. The 1-directional competition between S33 and mAbs S12 and S17 suggests that these mAbs recognized distinct epitopes that are close together, rather than overlapping, on the GP. Unfortunately, several attempts to identify the sequences of the conserved epitopes on the EBOV GP by using a phage display peptide library were not successful (data not shown).

Finally, an immunofluorescence assay (IFA) found that none of the mAbs detected EBOV infection in Vero cells by (data not shown). The absence of EBOV-specific staining by IFA was surprising, but the use of this technique under biosafety level 4 conditions requires fixation of EBOV-infected cells with 10% formalin for 48 hours before samples can be transferred to a biosafety level 2 facility and stained. This prolonged fixation has been shown to affect antigenicity.

Overall, these data show that conserved epitopes are present on the GPs of all known *Ebolavirus* species. While the antibodies described in this study did not display neutralizing activity, nonneutralizing antibodies have been shown to protect in vivo [7]. Therefore, future studies will include testing the in vivo efficacy of these mAbs to protect against lethal EBOV challenge.

In conclusion, we developed and performed an initial characterization of a panel of broadly reactive mAbs against *Ebolavirus* species. These mAbs will be crucial for the development of novel point-of-care diagnostic assays that can detect viral antigen during outbreaks of all known *Ebolavirus* species.

## Notes

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**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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