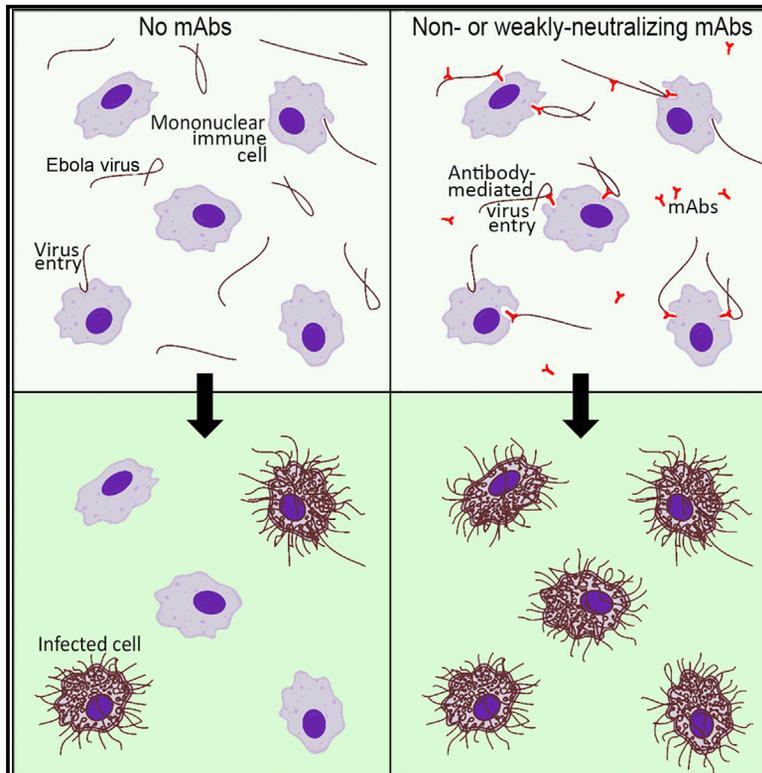


## Antibody-Dependent Enhancement of Ebola Virus Infection by Human Antibodies Isolated from Survivors

### Graphical Abstract



### Authors

Natalia A. Kuzmina, Patrick Younan, Pavlo Gilchuk, ..., Palaniappan Ramanathan, James E. Crowe, Jr., Alexander Bukreyev

### Correspondence

james.crowe@vanderbilt.edu (J.E.C.), alexander.bukreyev@utmb.edu (A.B.)

### In Brief

In this paper, Kuzmina et al. demonstrate that filovirus antibodies from human survivors present at low concentrations are capable of enhancement of infection, suggesting that low levels of antibodies in humans may facilitate virus spread. The enhancement can be caused by antibodies of various epitope specificities, neutralizing capacities, and subclasses.

### Highlights

- mAbs from filovirus human survivors tested for their ability to facilitate ADE
- ADE is not restricted to a particular epitope, neutralizing capacity, or subclass
- Lower-affinity interactions still cause ADE
- ADE counteracts antibody-mediated protection and facilitates infection



# Antibody-Dependent Enhancement of Ebola Virus Infection by Human Antibodies Isolated from Survivors

Natalia A. Kuzmina,<sup>1,2</sup> Patrick Younan,<sup>1,2</sup> Pavlo Gilchuk,<sup>5</sup> Rodrigo I. Santos,<sup>1,2</sup> Andrew I. Flyak,<sup>4,7</sup> Philipp A. Ilinykh,<sup>1,2</sup> Kai Huang,<sup>1,2</sup> Ndongala M. Lubaki,<sup>1,2</sup> Palaniappan Ramanathan,<sup>1,2</sup> James E. Crowe, Jr.,<sup>4,5,6,\*</sup> and Alexander Bukreyev<sup>1,2,3,8,\*</sup>

<sup>1</sup>Department of Pathology, University of Texas Medical Branch, Galveston, TX 77555, USA

<sup>2</sup>Galveston National Laboratory, Galveston, TX 77550, USA

<sup>3</sup>Department of Microbiology & Immunology, University of Texas Medical Branch, Galveston, TX 77555, USA

<sup>4</sup>Department of Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, TN 37232, USA

<sup>5</sup>Vanderbilt Vaccine Center, Vanderbilt University Medical Center, Nashville, TN 37232, USA

<sup>6</sup>Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN 37232, USA

<sup>7</sup>Present address: Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA

<sup>8</sup>Lead Contact

\*Correspondence: [james.crowe@vanderbilt.edu](mailto:james.crowe@vanderbilt.edu) (J.E.C.), [alexander.bukreyev@utmb.edu](mailto:alexander.bukreyev@utmb.edu) (A.B.)

<https://doi.org/10.1016/j.celrep.2018.07.035>

## SUMMARY

Some monoclonal antibodies (mAbs) recovered from survivors of filovirus infections can protect against infection. It is currently unknown whether natural infection also induces some antibodies with the capacity for antibody-dependent enhancement (ADE). A panel of mAbs obtained from human survivors of filovirus infection caused by Ebola, Bundibugyo, or Marburg viruses was evaluated for their ability to facilitate ADE. ADE was observed readily with all mAbs examined at sub-neutralizing concentrations, and this effect was not restricted to mAbs with a particular epitope specificity, neutralizing capacity, or subclass. Blocking of specific Fc $\gamma$  receptors reduced but did not abolish ADE that was associated with high-affinity binding antibodies, suggesting that lower-affinity interactions still cause ADE. Mutations of Fc fragments of an mAb that altered its interaction with Fc receptors rendered the antibody partially protective *in vivo* at a low dose, suggesting that ADE counteracts antibody-mediated protection and facilitates dissemination of filovirus infections.

## INTRODUCTION

The viral family *Filoviridae* includes several viruses that cause severe human diseases with high case fatality rates: Ebola (EBOV), Bundibugyo (BDBV), Sudan (SUDV), Marburg (MARV), and Ravn (RAVV) (Burk et al., 2016). Research during the devastating 2013–2016 outbreak of Ebola virus disease (EVD) in Western Africa highlighted the lack of understanding of fundamental characteristics of filovirus pathogenesis and

demonstrated an urgent need to develop countermeasures to combat future outbreaks.

Filoviruses have multiple mechanisms of immune evasion, including suppression of innate (Messaoudi et al., 2015) and adaptive (Lubaki et al., 2016) immunity. However, the relative contributions of these mechanisms to the inability of a host to control filovirus infections are unclear. Previous reports have suggested that antibodies may contribute to clearance of filovirus infections. During the 1995 outbreak of EVD in Kikwit, Democratic Republic of the Congo, immunoglobulin M (IgM) and IgG responses appeared in patients on days 8–10 after disease onset (Ksiazek et al., 1999). Survivors of the 1996 EVD outbreak in Gabon had greater antibody responses than those that succumbed (Baize et al., 1999). A detailed characterization of the immune status of four EVD patients from the 2013–2016 epidemic in Western Africa performed at Emory University demonstrated that they developed both IgM and IgG responses during the second week of illness (McElroy et al., 2015). These data suggest that the appearance of antibodies correlates with, and may contribute to, clearance of filovirus infections. This role for antibodies in clearance of acute infections may pertain to other infections. A recent study that modeled antibody dynamics during primary dengue virus infections in 53 patients suggested that antibodies play a key role in clearance of the virus (Clapham et al., 2016). Some features of murine monoclonal antibodies (mAbs) specific for filoviruses suggest that the role of the antibody response in filovirus clearance is complex. For example, several murine mAbs specific to EBOV (Takada et al., 2007) or MARV (Nakayama et al., 2011) and human plasma from EVD survivors (Takada et al., 2003) caused enhancement of infection *in vitro*. This phenomenon, known as antibody-dependent enhancement (ADE), has been demonstrated for dengue viral infections (Acosta and Bartenschlager, 2016) and also has been demonstrated for HIV-1 (Tay et al., 2016). The high lethality and sporadic nature of filovirus infections prevented investigation of the relevance of ADE for filoviruses;



however, some studies with non-human primates suggest this possibility. Treatment of four EBOV-infected macaques with convalescent serum from EBOV-immune macaques failed to protect animals and resulted in viral titers at the time of death or moribund condition  $\sim$ 100-fold greater than those in the control animals that did not receive immune serum (Jahrling et al., 2007). Moreover, passive transfer of mAb KZ52 to four rhesus macaques completely failed to affect the course of infection in three macaques and significantly delayed death of one macaque. Importantly, in organs harboring the greatest amounts of virus (liver, spleen, kidney, and lungs), virus loads in the former three animals were greater than those in the control animal (Oswald et al., 2007). Eventually, protection by extremely high doses of polyclonal antibodies (Dye et al., 2012) and mAbs (Qiu et al., 2012) was achieved. While the complete failure of the EBOV treatments with antibody doses expected to be protective and the increased virus loads in organs of recipient animals suggest the possibility of ADE, it has never been demonstrated that antibodies isolated from survivors of natural human EBOV infections can mediate ADE.

Recently, dramatic progress has been achieved in isolation and characterization of mAbs from human survivors of EVD caused by infections with EBOV, BDBV, or from a human survivor of MARV disease (MVD), some of which demonstrated impressive protective efficacy *in vivo* (Bornholdt et al., 2016; Corti et al., 2016; Flyak et al., 2015, 2016; Mire et al., 2017). However, it is unknown whether any of these mAbs also may cause ADE. Investigation of this possibility could be important for the rational selection of therapeutic mAbs, doses, and treatment regimens.

Monocytes, macrophages and dendritic cells (DCs) are potent antigen-presenting cells that initiate the adaptive immune response. These cells also are involved in antibody-dependent phagocytosis of viral particles, as demonstrated for influenza A virus (Ana-Sosa-Batiz et al., 2016) and HIV-1 (Ana-Sosa-Batiz et al., 2014). On the other hand, DCs are among the early targets of infection by filoviruses (Geisbert et al., 2003) and likely contribute to dissemination of the infection throughout tissues and organs of the host. Similarly, monocytes, macrophages, and DCs may be involved in ADE during natural human dengue infections (Acosta and Bartenschlager, 2016). In the present study, we investigated the effects of a diverse panel of mAbs derived from survivors of EVD caused by natural EBOV or BDBV infections or from a survivor of MVD in multiple types of immune cells from the monocyte-macrophage lineage. We found that non-neutralizing antibodies can enhance infection, and also that neutralizing mAbs, when added at sub-neutralizing concentrations, exhibit ADE capacity. This enhancing effect was independent of antibody epitope specificity, neutralizing potency or IgG subclass.

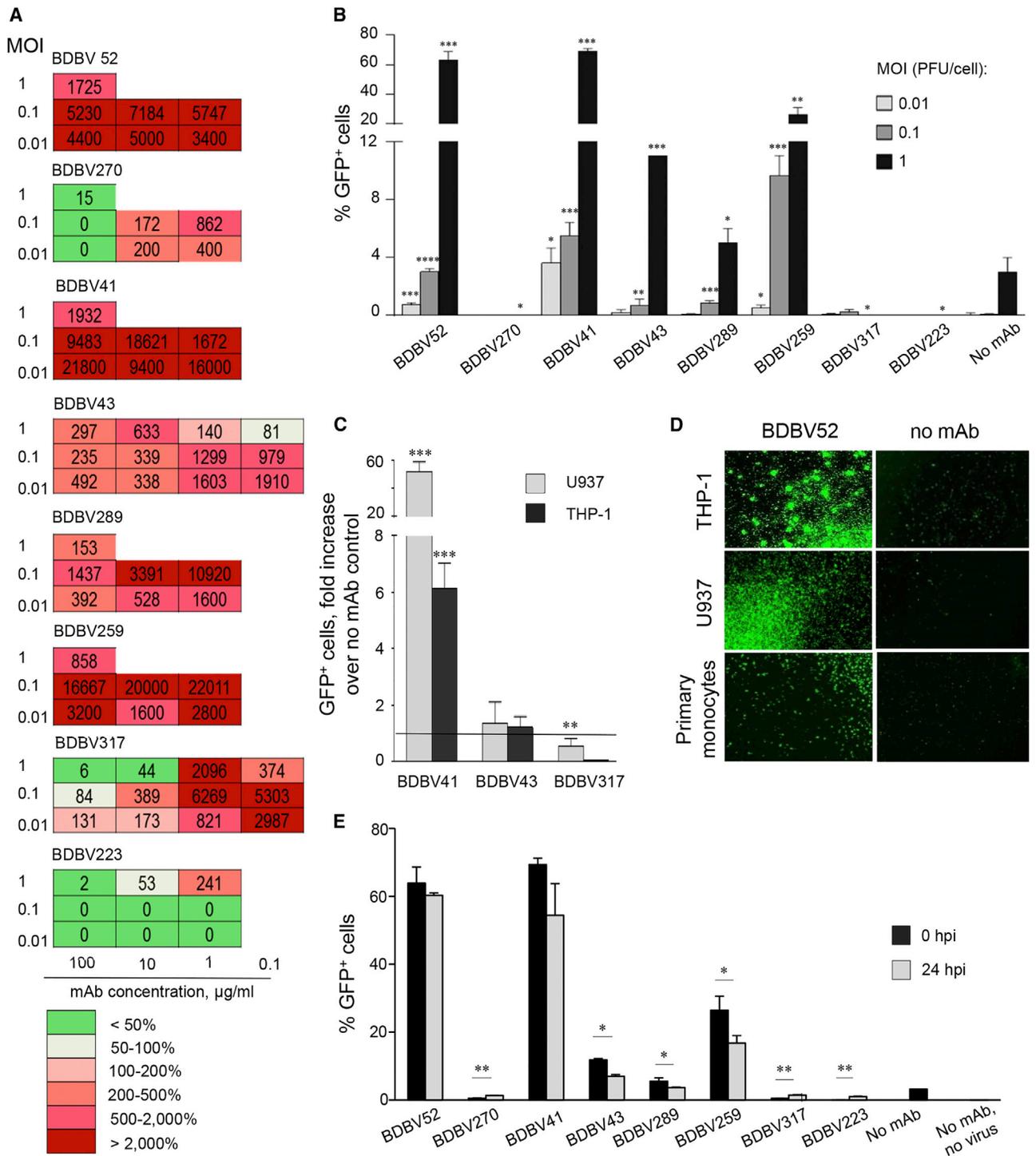
## RESULTS

### ADE of Filovirus Infections Can Be Caused by Antibodies of Different Epitope Specificities, Different Neutralizing Capacities, and Different Subclasses

In previous studies, we isolated large and diverse panels of mAbs from human survivors of EVD caused by BDBV (Flyak

et al., 2016), EBOV (P.G., N.K., P.A.I., K.H., A.I.F., A.B., J.E.C., B.M. Gunn, A. Bryan, E. Davidson, B.J. Doranz, H.L. Turner, M.L. Fusco, M.S. Bramble, N.A. Hoff, E. Binshtein, N. Kose, R. Flinko, C. Orlandi, R. Carnahan, E.H. Parrish, A.M. Sevy, R.G. Bombardi, P.K. Singh, P. Mukadi, J.J. Muyembe-Tamfum, M.D. Ohi, E. Ollmann Saphire, G.K. Lewis, G. Alter, A.B. Ward, and A.W. Rimon, unpublished data) and from a survivor of MVD (Flyak et al., 2015) specific for the viral glycoprotein (GP), the only envelope protein of filoviruses (Lee et al., 2008). Most of these mAbs can be segregated into two groups: (1) those binding to the receptor-binding domain (RBD) and glycan cap area located at the N-terminal part of GP1 and (2) those binding to the membrane proximal external region (MPER) and internal fusion loop of GP2 (Flyak et al., 2015, 2016, 2018). To test whether filovirus antibodies from human survivors can cause ADE, we selected mAbs that recognize diverse epitopes located either in the RBD/glycan cap or MPER. The choice of mAbs was based on differences in epitopes, ability to neutralize BDBV and heterologous ebolaviruses *in vitro*, levels of protective efficacy *in vivo*, and IgG subclasses (Flyak et al., 2016) (Figures S1A and S1B). To monitor viral infection, we used a chimeric filovirus carrying GP of BDBV and the rest of the proteins of EBOV (EBOV/BDBV-GP), and expressing the enhanced GFP (eGFP) from an added gene (Ilinykh et al., 2016). We treated the monocytic cell line THP-1 with the selected panel of mAbs at concentrations 100, 10, 1, or 0.1  $\mu$ g/mL and inoculated them at a multiplicity of infection (MOI) of 1.0, 0.1, or 0.01 plaque-forming unit (PFU)/cell (as determined by virus titration in Vero E6 cell monolayers). The cells were incubated for 48 hr, and the numbers of infected (eGFP<sup>+</sup>) cells were quantified by flow cytometry (Figures 1A and 1B). Strikingly, we detected a reduction in the number of eGFP<sup>+</sup> cells only with the highest concentrations of BDBV270 and BDBV223 mAbs. In all other concentrations, and for each of the eight mAbs tested, we observed a strong increase in numbers of infected cells, suggesting enhancement of the infection. Comparison of the effects of mAb concentrations and viral doses demonstrated an ADE that was generally greater at lower (sub-neutralizing) concentrations of mAbs and greater viral doses, although direct correlations were not observed as each mAb exhibited its own optimal neutralizing concentration. These data indicate that enhancement of infection occurs under conditions of incomplete virus neutralization.

The most pronounced ADE was detected with two of the mAbs specific to the glycan cap region (BDBV52 and BDBV41), the mAb targeting the internal fusion loop (BDBV259), and the MPER-specific mAb BDBV317 (Figure 1A). The other glycan cap-specific mAb BDBV270 and the MPER-specific mAb BDBV223 demonstrated only very moderate levels of enhancement. BDBV41, BDBV43, and BDBV289 target the same part of the GP, based on sequence analysis of the GP of escape mutants (Figure S1A) and have comparable 50% maximal inhibitory concentrations (IC<sub>50</sub>). However, the enhancement caused by BDBV41 (1.9- to 21.8-fold increase in the number of infected cells compared to the no antibody control) was much greater than that caused by the two other mAbs. The most potent neutralizing mAb BDBV223 demonstrated the least ADE capacity. These data indicate that ADE of ebolavirus infection does not depend significantly on the epitope specificity of mAb.



**Figure 1. ADE of EBOV/BDBV-GP Infection in Primary Monocytes or Monocytic Cell Lines**

(A) Heatmaps showing percentages of eGFP<sup>+</sup> THP-1 cells at differing concentrations of virus or mAbs, normalized to cells with no mAbs added (100%).

(B) Percentages of eGFP<sup>+</sup> THP-1 cells at differing viral doses in the presence of mAbs at 100  $\mu\text{g/ml}$ .

(C) Fold increase over no mAb control of eGFP<sup>+</sup> THP-1 and U937 cells infected at MOI of 1.0 PFU/cell and treated with 100  $\mu\text{g/ml}$  of the indicated mAb.

(D) Aggregation of the indicated cells inoculated at an MOI of 1.0 PFU/cell and treated with 100  $\mu\text{g/ml}$  of BDBV52 mAb.

(E) Percentages of eGFP<sup>+</sup> THP-1 cells infected at an MOI of 1.0 PFU/cell and treated with the indicated mAb at 100  $\mu\text{g/ml}$  immediately after infection (0 hpi) or at 24 hpi.

(B), (C), (E), mean values  $\pm$ SD based on triplicate samples. Differences to no antibody control: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$  (Multiple t test).

Since the percentage of eGFP<sup>+</sup> cells was used as a measure of ADE, it was important to confirm that mAb-mediated increase of eGFP<sup>+</sup> monocyte numbers results in a higher level of infectious viral particles released from infected cells (that is, enhanced infection). However, the presence of neutralizing mAbs in the medium could interfere with accurate quantification of viable virions. Therefore, we selected a few representative mAbs with differing ADE levels demonstrated in Figure 1B (BDBV52, BDBV41, and BDBV43), inoculated THP-1 cells with virus/mAb mixtures, incubated for 1 hr, washed cells with PBS, resuspended them in fresh medium with no mAbs added, and determined the virus titers in cell supernatants at 48 hr after infection (Figure S2). Indeed, the numbers of virions released from the monocytes infected in presence of filovirus-specific mAbs were 16.7- to 54.6-fold higher than in the no mAb control, with no significant difference observed between the irrelevant 2D22 mAb specific for dengue virus (Fibriansah et al., 2015) and the no mAb groups.

To supplement the data obtained in THP-1 cells, we tested ADE in an alternative monocytic cell line, U937. Cells were inoculated with EBOV/BDBV-GP at an MOI of 1.0 PFU/cell and treated with mAbs BDBV41, BDBV43, or BDBV317. Again, the mAbs induced ADE, which was even greater than that observed in THP-1 cells. The greatest increase was detected for mAb BDBV41 and resulted in a 52-fold increase in the percentage of eGFP<sup>+</sup> cells (Figure 1C). Interestingly, addition of the ADE-inducing mAb BDBV52 to infected THP-1, U937, or primary human monocytic cells resulted in the formation of large aggregates of infected cells (Figure 1D).

Next, we tested whether delayed treatment with mAbs also induced ADE. THP-1 cells were inoculated with EBOV/BDBV-GP and treated with each of the eight selected BDBV mAbs, either immediately after addition of the virus or 24 hr later. The delayed treatment also induced ADE, which was only marginally lower than that following treatment applied immediately after infection (Figure 1E).

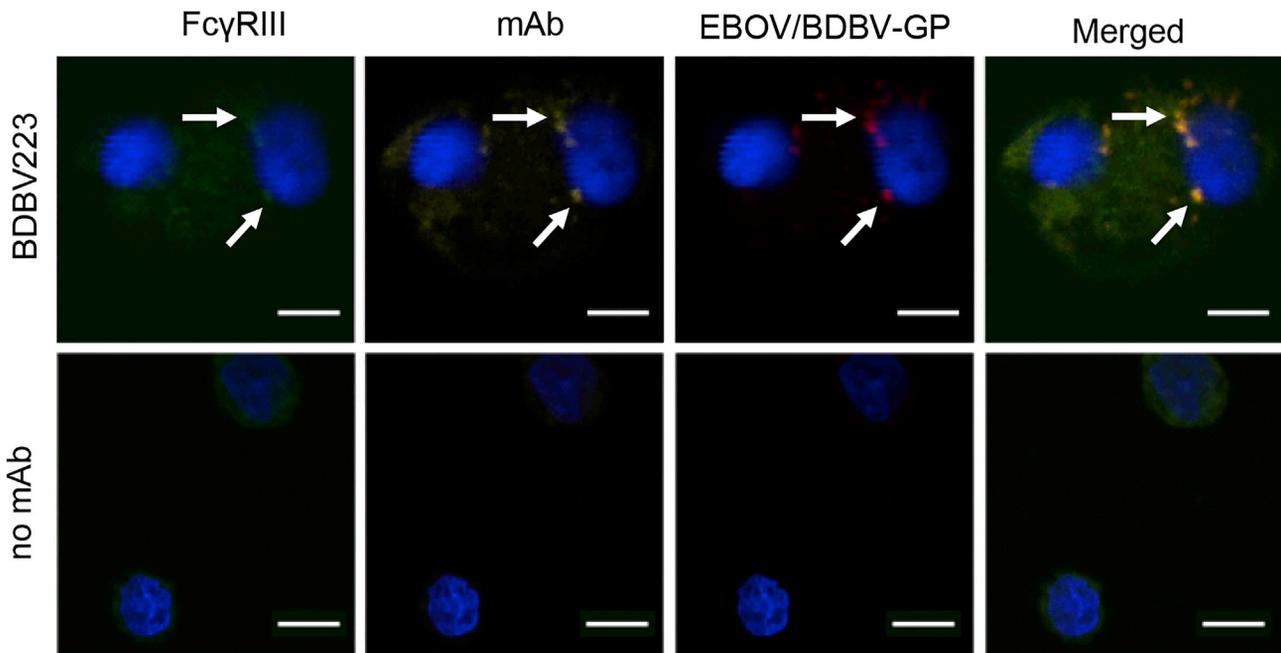
We next sought to determine whether filovirus mAbs isolated from survivors induced ADE of infections caused by multiple filoviruses. First, we tested a panel of mAbs recovered from an EVD survivor of the 2013–2016 epidemic for the ability to induce ADE of EBOV infection. These mAbs neutralized EBOV at varying IC<sub>50</sub> (Figure S3A). The epitopes of EBOV-87, EBOV-141, and EBOV-333 are located in the N-terminal 300 amino acids of GP, while that of EBOV-68 and EBOV-91 are in the other part of GP, based on the ability of these mAbs to bind to secreted GP (sGP) that shares part of its sequence with the N-terminal part of GP (Sanchez et al., 1996; Volchkov et al., 1995). We were able to test a new multifunctional neutralizing mAb EBOV-520 (P.G., N.K., P.A.I., K.H., A.I.F., A.B., J.E.C., B.M. Gunn, A. Bryan, E. Davidson, B.J. Doranz, H.L. Turner, M.L. Fusco, M.S. Bramble, N.A. Hoff, E. Binshtein, N. Kose, R. Flinko, C. Orlandi, R. Carnahan, E.H. Parrish, A.M. Sevy, R.G. Bombardi, P.K. Singh, P. Mukadi, J.J. Muyembe-Tamfum, M.D. Ohi, E. Ollmann Saphire, G.K. Lewis, G. Alter, A.B. Ward, and A.W. Rimon, unpublished data), which binds in the region near the internal fusion loop (IFL) but in an unusual binding pose that alters receptor bind-

ing indirectly. We inoculated THP-1 cells with a recombinant eGFP-expressing EBOV at an MOI of 1.0 PFU/cell and treated them with mAbs at 100 μg/mL. Only mAbs EBOV-333 and EBOV-520 slightly reduced the percentage of infected cells, but not significantly, while all other mAbs demonstrated ADE ranging from a 2.7- to 15.2-fold increase in infected cells (Figure S3B). We extended testing of this mAb panel with the well-characterized EBOV-specific murine-human chimerized mAbs 13C6 and 2G4, which are predecessors of the components of the ZMapp cocktail (Olinger et al., 2012; Qiu et al., 2011, 2016; Wilson et al., 2000) and bind to glycan cap and GP base, respectively (Davidson et al., 2015; Murin et al., 2014). Surprisingly, non-neutralizing 13C6 mAb caused a 50-fold enhancement of infection when added at 100 μg/mL, whereas mAb 2G4 demonstrated a strong neutralizing effect (Figures S3A and S3B).

Further, we tested ADE of SUDV infection. We selected several mAbs from EVD survivors that neutralized multiple ebolaviruses including SUDV: BDBV43 used in our previous experiments (Figure 1), BDBV324 (Flyak et al., 2016), EBOV-293 (unpublished data), and EBOV-520 (P.G., N.K., P.A.I., K.H., A.I.F., A.B., J.E.C., B.M. Gunn, A. Bryan, E. Davidson, B.J. Doranz, H.L. Turner, M.L. Fusco, M.S. Bramble, N.A. Hoff, E. Binshtein, N. Kose, R. Flinko, C. Orlandi, R. Carnahan, E.H. Parrish, A.M. Sevy, R.G. Bombardi, P.K. Singh, P. Mukadi, J.J. Muyembe-Tamfum, M.D. Ohi, E. Ollmann Saphire, G.K. Lewis, G. Alter, A.B. Ward, and A.W. Rimon, unpublished data). First three mAbs bind to the same antigenic site within the glycan cap of ebolavirus GP, which is well conserved among ebolaviruses but EBOV-520 binds to a conformational epitope in the base region. All mAbs neutralized EBOV, BDBV, and SUDV in Vero E6 cells at a concentration of 10 μg/mL (Flyak et al., 2016; unpublished data). Testing these mAbs at the same concentration in THP-1 cells inoculated with the chimeric EBOV carrying SUDV GP (EBOV/SUDV-GP) resulted in a 2.3- to 5.6-fold increase in the percentage of infected (eGFP<sup>+</sup>) cells, except EBOV-520, which significantly reduced infection at this concentration (Figure S3C).

To extend the study to MARV, we used a panel of five mAbs isolated from a MVD survivor: MR72, MR114, MR144, MR191, or MR228, which belong to different competition-binding groups on MARV GP and differ in their neutralizing capacity (Flyak et al., 2015). When THP-1 cells inoculated with a chimeric filovirus carrying MARV GP (EBOV/MARV-GP) at an MOI of 1.0 PFU/cell were treated with the mAbs at 100 μg/mL, three out of five mAbs reduced the infection (Figure S3D). In contrast, treatment with the same mAbs at a sub-neutralizing concentration of 1 μg/mL resulted in ADE, ranging from a 2.4- to 6.0-fold increase in the percentage of infected (eGFP<sup>+</sup>) cells. The only exception was mAb MR114, which has a very limited binding potency (Flyak et al., 2015); this antibody neither induced ADE nor neutralized the virus.

These data suggest that mAbs from human EVD or MVD survivors possess ADE capacity against both homologous and heterologous filovirus species recognized by these mAbs. Furthermore, the effect did not depend significantly on the epitope specificity of mAbs, the neutralizing capacity, any specific antibody subclass, or the time of antibody treatment.



**Figure 2. Aggregation of Virus Particles with mAbs and Co-localization of Aggregates with FcRs**

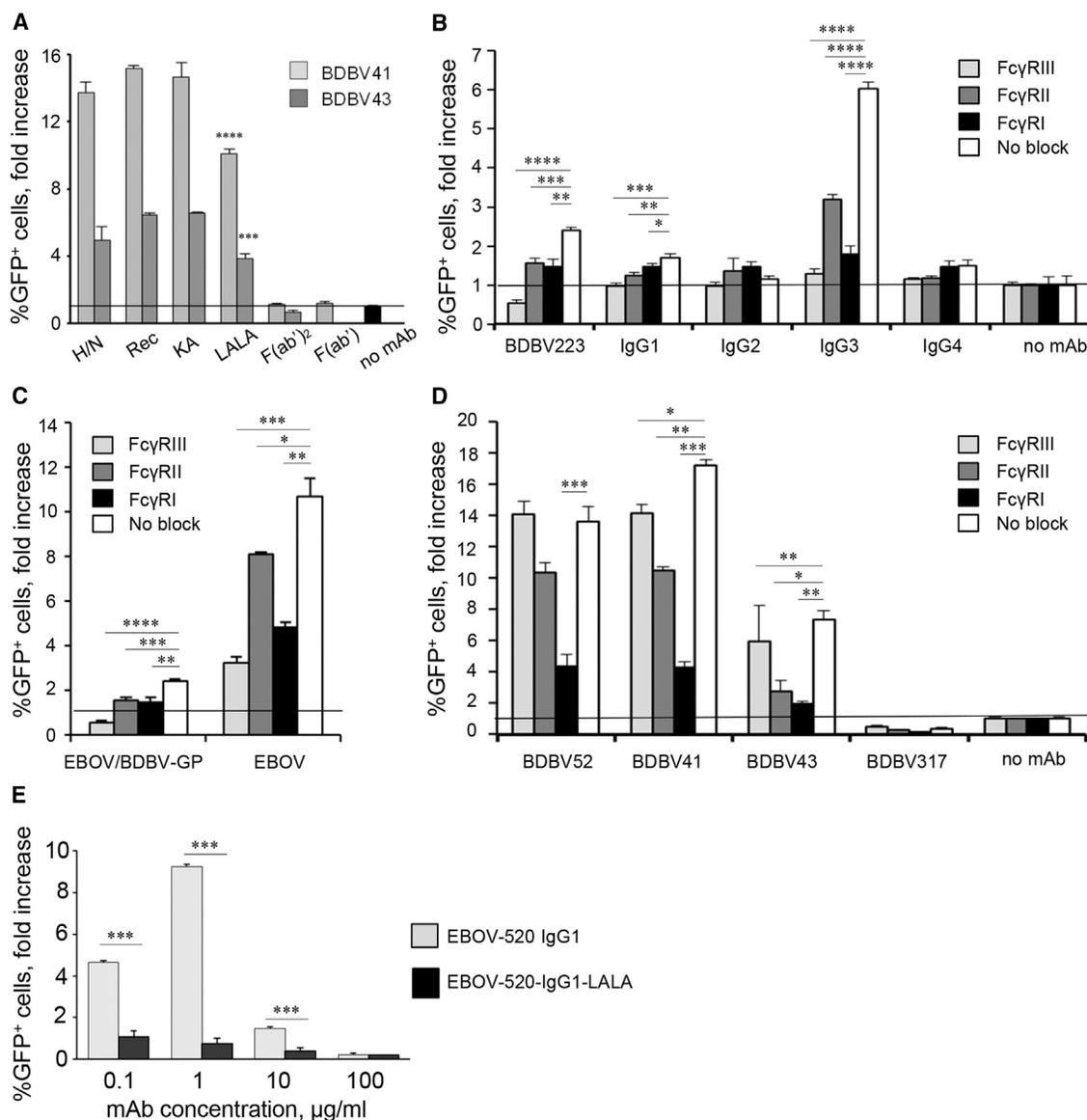
Confocal microscopy of THP-1 cells inoculated with EBOV/BDBV-GP in the presence of BDBV223 mAb (top panel) or in the absence of mAb (bottom panel), 1 hr after inoculation. Fc $\gamma$ RIII is stained with antibodies labeled with Alexa Fluor 488 (green), mAbs with Dy594 (orange), or the virus with Alexa Fluor 647 (red). Scale bar, 5  $\mu$ m. Nuclei are stained with DAPI (blue). Arrows point to co-localization of Fc $\gamma$ Rs, mAb, and virus.

### ADE Depends on the Presence of Fc Fragments and Fc $\gamma$ Receptors and Is Induced Preferentially by Their High-Affinity Interactions

It is generally thought that certain pathogens may benefit from ADE of infection when antibodies bind to virions resulting in enhanced entry into immune cells bearing Fc receptors (FcRs). The process is mediated by interactions of Fc $\gamma$ Rs with the antibody Fc region (Swisher and Feldman, 2015). We inoculated THP-1 cells with EBOV/BDBV-GP with or without the mAb BDBV223 and incubated for 1 hr; we observed aggregation of viral particles and co-localization of aggregates with Fc receptors in presence of BDBV223 (Figure 2). To test the involvement of Fc regions in the observed ADE of filovirus GP-reactive human mAbs, we evaluated enhancing properties of several mAbs with their Fc fragments removed. Incubation of infected cells with F(ab') or F(ab')<sub>2</sub> fragments of BDBV41 or BDBV43 did not cause ADE (Figures 3A and S4A). Next, we tested whether the observed ADE depends on a certain IgG subclass. In the panel of mAbs selected for testing, BDBV223 was of the IgG3 subclass, while the other mAbs were IgG1 molecules. We constructed recombinant IgG1, IgG2, IgG3, or IgG4 subclass forms of mAb BDBV223. This mAb neutralized EBOV/BDBV-GP effectively when engineered to contain the Fc of any of the subclasses, with IgG2 and IgG3 being the most potent against EBOV (Table S1). We tested the Fc-modified mAbs for the ability to induce ADE at concentrations of 0.1 or 1  $\mu$ g/mL in THP-1 cells inoculated with EBOV/BDBV-GP or EBOV at an MOI of 1.0 PFU/cell. Antibody of the IgG3 subclass induced high-level ADE at both concentrations tested with both viruses, while antibody of

the other subclasses induced much lower (IgG1, IgG4) or no (IgG2) ADE (Figures S4A and S4B). When cells were inoculated with EBOV, which is less sensitive to neutralization by BDBV223 (Figure S4B), ADE was observed with the IgG1 subclass. Both the hybridoma-derived native BDBV223 IgG3 and its recombinant IgG3 form induced a much greater level of ADE with EBOV (50-fold) than with EBOV/BDBV-GP (2- to 8-fold) (Figures S4A and S4B), which is likely related to a lesser affinity of this mAb for binding to the EBOV GP. The ADE effect was greater for the recombinant IgG3 than for the hybridoma-produced antibody, presumably due to a difference in glycosylation of asparagine 297 in the Fc domain of the heavy chain (Borrok et al., 2012), which is known for its heterogeneity (Higel et al., 2016), or another glycosylated amino acid residue.

We examined the effects of blocking each of the three types of Fc $\gamma$  receptors, Fc $\gamma$ RI, Fc $\gamma$ RII, or Fc $\gamma$ RIII, on ADE. THP-1 cells were inoculated with FcR-blocking antibodies for 1 hr, treated with recombinant BDBV223 IgG1, IgG2, IgG3, or IgG4 mAbs at a concentration 0.1  $\mu$ g/mL, and inoculated with EBOV/BDBV-GP at an MOI of 1.0 PFU/cell (Figure 3B). As recombinant IgG1, IgG2, or IgG4 did not cause ADE, blocking of any of the receptors resulted in little or no effect. In contrast, when IgG3 was used, blocking of Fc $\gamma$ RIII eliminated ADE, while blocking of Fc $\gamma$ RI or Fc $\gamma$ RII significantly reduced it. Testing of hybridoma-produced BDBV223 antibody with EBOV, which is less sensitive to this mAb (Figure S1B), also demonstrated the greatest reduction of ADE by blocking of Fc $\gamma$ RIII and much lesser effects by blocking of Fc $\gamma$ RI or Fc $\gamma$ RII (Figure 3C). These data are surprising, because a single THP-1 cell expresses Fc $\gamma$ RI



**Figure 3. ADE Depends on the Presence of Fc Receptors, Fc Fragments, and on the Subclass of IgG: Fold Increases of the Percentages of Infected (eGFP<sup>+</sup>) THP-1 Cells Treated with the Indicated Filovirus mAb with or without FcγR-Blocking Antibodies, over Cells Not Treated with Filovirus mAbs**

In all experiments, MOI of 1 PFU/cell was used.

(A) Cells inoculated with EBOV/BDBV-GP and treated with 10 μg/mL of various forms of BDBV41 or BDBV43: H, hybridoma derived BDBV41; N, plant-derived BDBV43; KA and LALA, modifications of Fc fragments; F(ab')<sub>2</sub> fragments generated by pepsin digestion; F(ab') fragments generated by papain digestion.

(B) Cells infected with EBOV/BDBV-GP and treated with 0.1 μg/mL of hybridoma-produced BDBV223 or recombinant forms of BDBV223 with differing subclasses, in the presence of FcγR-blocking antibodies.

(C) Cells inoculated with the indicated viruses and treated with 0.1 μg/mL BDBV223 in presence of the indicated FcγR-blocking antibodies.

(D) Cells inoculated with EBOV/BDBV-GP and treated with the indicated mAbs (10 μg/mL) in the presence of the indicated FcγR-blocking antibodies.

(E) Cells infected with EBOV-eGFP at MOI of 1.0 PFU/cell and treated with the indicated concentrations of EBOV-520 or EBOV-520-LALA.

Mean values ± SD based on triplicate samples. Differences to no antibody control (A) or for the indicated pairs: \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001 (multiple t test).

with ~35,000 high-affinity binding sites for IgG1 and ~50,000 low-affinity FcγRII for IgG2 on the cell surface (Fleit and Kobasiuk, 1991), but expression of FcγRIII in these cells is limited and was demonstrated only recently (Tay et al., 2016). Interest-

ingly, unlike these data, dengue ADE is associated primarily with FcγRI and FcγRII (Acosta and Bartenschlager, 2016; Littau et al., 1990). The remaining BDBV mAbs used in these experiments were of the IgG1 subclass. In contrast to BDBV223,

a significant inhibition of ADE for these mAbs was observed with blocking of Fc $\gamma$ RI, but not Fc $\gamma$ RII or Fc $\gamma$ RIII (Figure 3D), which is consistent with the highest binding affinity of Fc $\gamma$ RI for IgG1 (Gillis et al., 2014).

We also evaluated the effect of antibody Fc region mutations L234A and L235A (LALA) (Reusch and Tejada, 2015), which reduce antibody binding to Fc $\gamma$ RIIIa and Fc $\gamma$ RIIIb but not Fc $\gamma$ RI (Hessell et al., 2007), on the induction of ADE. Introduction of the mutations in mAbs BDBV41 and BDBV43, which are IgG1, only slightly reduced ADE (Figure 3A). These data are consistent with the preferential binding of IgG1 to Fc $\gamma$ RI. The LALA mutation of EBOV-520 (IgG1 subclass as well) abrogated ADE *in vitro* even more efficiently (Figures 3E and S4C, see below). Next, we tested the effect of the Fc region mutation K322A (KA), the residue that is critical for antibody-dependent complement activation (Thommesen et al., 2000). We did not observe an effect for this mutation (Figure 3A), suggesting that complement does not play a role in the observed ADE.

Taken together, these data indicate that ADE of filovirus infections involves interaction of Fc receptors with Fc domains of antibodies. The data also demonstrate that blocking of an Fc $\gamma$ R preferentially interacting with an antibody subclass that causes ADE significantly reduces but does not completely eliminate the ADE. This suggests that low-affinity interactions between antibodies and Fc $\gamma$ Rs also may contribute ADE, albeit at a lesser extent.

### Induction of ADE in Primary Human Immune Cells

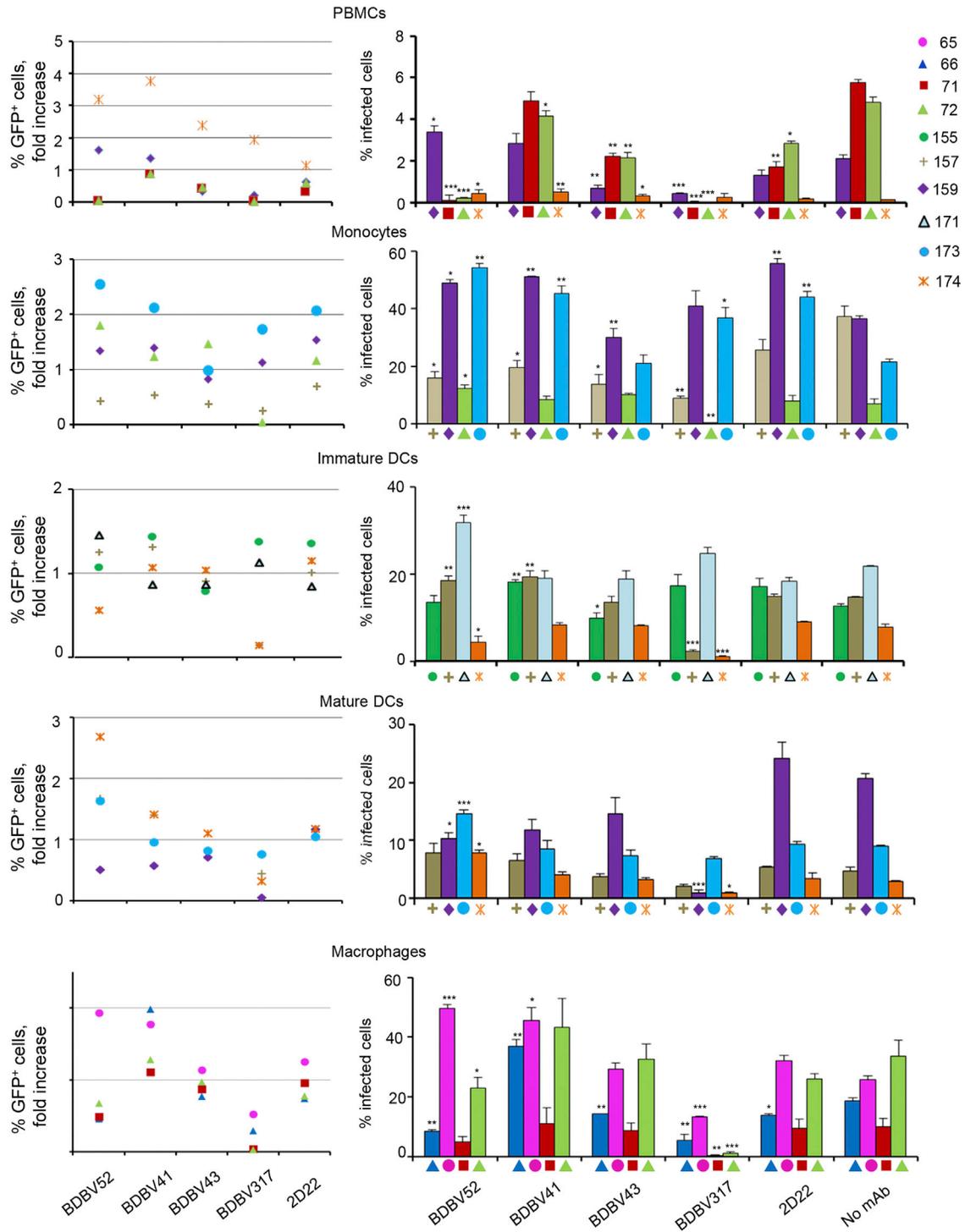
As cell lines of tumor origin may exhibit different physiologic and expression patterns compared to primary cells, we evaluated ADE in total peripheral blood mononuclear cells (PBMCs) and various myeloid cell types, including monocytes, immature DCs, mature DCs, and macrophages obtained from human donors and differentiated *in vitro* (Figure 4). These cells differ in their expression patterns of Fc receptors, which change during their differentiation and maturation. Each of these cell types supported EBOV/BDBV-GP infection but at very different levels and with a high donor-to-donor variability. mAbs caused variable ADE of an infection in each of the cell types obtained from many but not all donors. The PBMC samples revealed low susceptibility to infection, ranging from 0.2% to 6% of infected (eGFP<sup>+</sup>) cells, which reflects the relatively low percentages of putative filovirus-susceptible target cells present (e.g., monocytes) and perhaps viral uptake by non-susceptible cells expressing Fc receptors. Analysis of ADE in these cells demonstrated an increase (up to 3.8-fold) in the percentages of infected cells, compared to the no antibody control, for some donor-mAb combinations. In contrast, primary monocytes were more susceptible to infection, with the proportion of eGFP<sup>+</sup> cells ranging from 7% to 37% between donors. The ADE observed in these cells was comparable in magnitude to that in PBMCs (Figure 4). However, taking into consideration the higher absolute levels of infection in primary monocytes, this magnitude of enhancement may drastically increase the amount of virus circulating in blood of infected patients. Immature or mature DCs demonstrated similar susceptibility to EBOV infection (3%–22% or 3%–21% infected cells, respectively, as in our previous study, (Lubaki et al., 2013), which is somewhat lower than that observed for mono-

cytes. Both types of DCs showed induction of ADE at a lower level compared to that in PBMCs or monocytes. Macrophages demonstrated a high susceptibility to infection with 10%–32% of infected cells, which is comparable to monocytes, and ADE was observed at levels comparable to those in immature or mature DCs. To test whether the virus produced in primary immune cells may transfer infection to other cells efficiently, we inoculated PBMCs with EBOV/BDBV-GP at an MOI of 1.0 PFU/cell for 24 hr, washed them twice with phosphate-buffered saline (PBS), and placed the infected cells on top of uninfected Vero E6 cell monolayers. The plates were incubated for 48 hr, then PBMCs were removed, Vero E6 monolayers were washed with PBS and trypsinized, and the proportion of eGFP<sup>+</sup> Vero E6 cells was measured by flow cytometry. Vero E6 cells incubated with infected PBMCs from three donors showed the percentages of infected (eGFP<sup>+</sup>) cells ranging from 45% to 67% (Figure S5). This additional line of evidence shows that ADE caused by mAbs in immune cells may enhance the virus dissemination to the other cells.

These data demonstrate that human mAbs facilitate ADE in primary human immune cells at levels that vary greatly depending on the cell type, with the highest level in monocytes. Moreover, infected PBMCs efficiently transmitted infection to non-immune cells, suggesting that ADE may contribute to enhancement of virus spread through tissues and organs.

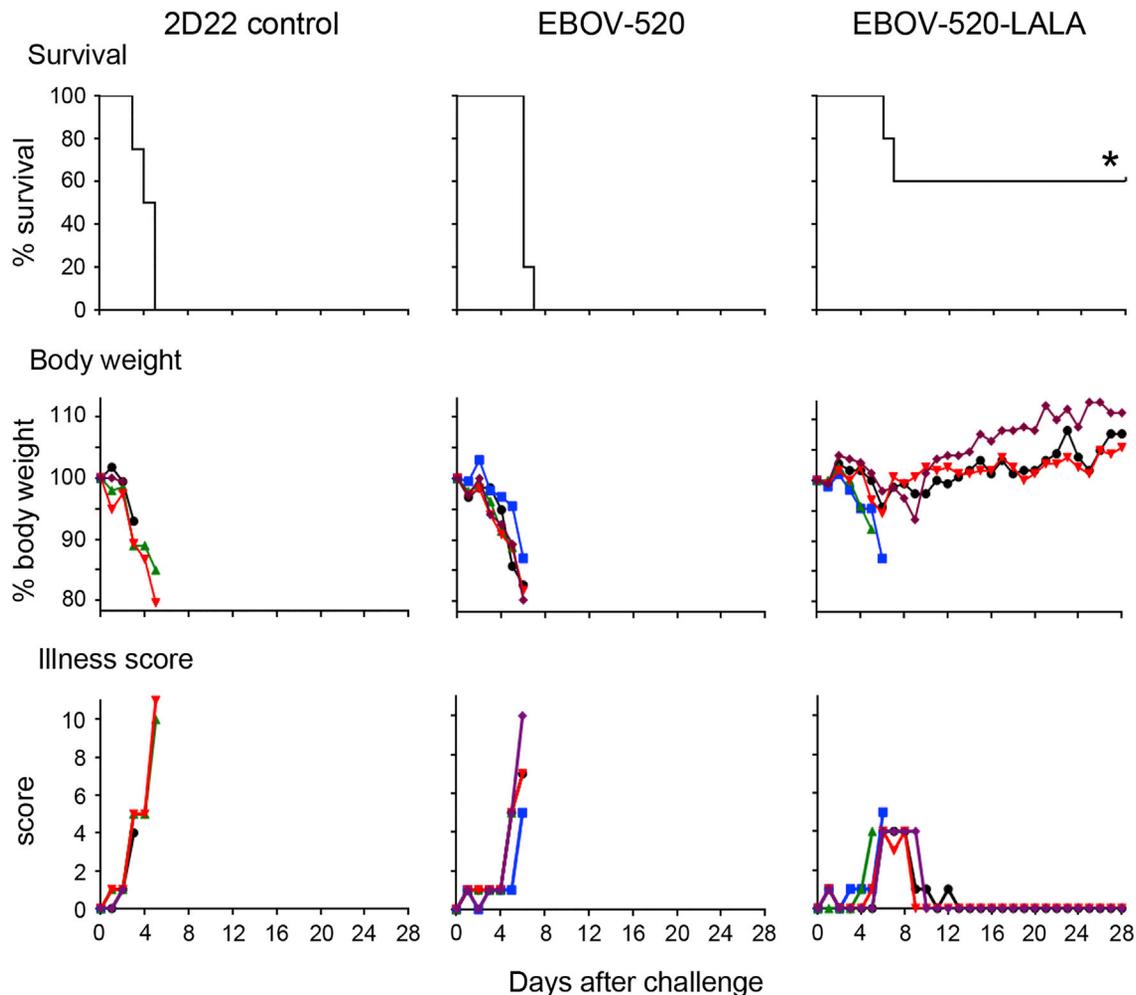
### An Antibody with Mutated Fc Domain, but Not the Original Antibody, Protects Laboratory Mice from EBOV Infection

There is no mouse model for BDBV, and many of the BDBV mAbs in the panel do not neutralize EBOV. Therefore, to address the physiological relevance of the observed ability of mAbs to enhance EBOV infection *in vivo*, we studied in more detail the mAb EBOV-520, which was isolated from a human survivor of the 2013–2016 EBOV epidemic in Western Africa in our recent study (P.G., N.K., P.A.I., K.H., A.I.F., A.B., J.E.C., B.M. Gunn, A. Bryan, E. Davidson, B.J. Doranz, H.L. Turner, M.L. Fusco, M.S. Bramble, N.A. Hoff, E. Binshtein, N. Kose, R. Flinko, C. Orlandi, R. Carnahan, E.H. Parrish, A.M. Sevy, R.G. Bombardi, P.K. Singh, P. Mukadi, J.J. Muyembe-Tamfum, M.D. Ohi, E. Ollmann Saphire, G.K. Lewis, G. Alter, A.B. Ward, and A.W. Rimon, unpublished data). One hundred micrograms of the mAb uniformly protects laboratory mice against lethal EBOV infection when administered 24 hr after challenge (P.G., N.K., P.A.I., K.H., A.I.F., A.B., J.E.C., B.M. Gunn, A. Bryan, E. Davidson, B.J. Doranz, H.L. Turner, M.L. Fusco, M.S. Bramble, N.A. Hoff, E. Binshtein, N. Kose, R. Flinko, C. Orlandi, R. Carnahan, E.H. Parrish, A.M. Sevy, R.G. Bombardi, P.K. Singh, P. Mukadi, J.J. Muyembe-Tamfum, M.D. Ohi, E. Ollmann Saphire, G.K. Lewis, G. Alter, A.B. Ward, and A.W. Rimon, unpublished data). As the original mAb is of the IgG4 subclass, we generated a recombinant IgG1 form of the mAb in order to increase the affinity of binding to Fc $\gamma$ Rs (Bournazos and Ravetch, 2017). Two recombinant forms of the IgG1 subclass of the mAb were produced: the non-mutated (wild-type) and a variant IgG1 derivative with L234A/L235A (LALA) mutations in the Fc fragment to reduce FcR binding. As noted above, EBOV-520 IgG1, but not EBOV-520 IgG1/LALA, caused ADE in EBOV-infected THP-1



**Figure 4. ADE of EBOV Infection in Primary Human Immune Cells**

Left, fold increase in percentage of infected (eGFP<sup>+</sup>) cells due to the presence of the filovirus mAb indicated on the x axis. Right, percentage of infected (eGFP<sup>+</sup>) cells in the presence or absence of filovirus mAbs indicated on the x axis, mean values  $\pm$  SD based on triplicate samples. Cells from individual donors are indicated with various symbols. Differences to no antibody control (multiple t test): \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



**Figure 5. ADE Counteracts Antibody-Mediated Protection *In Vivo***

EBOV-520 IgG1/LALA, but not EBOV-520 IgG1, protects laboratory mice from EBOV infection at a low dose. Kaplan-Meier survival curves, body weight, and illness score curves are shown. \*Survival difference between the EBOV-520 IgG1 and EBOV-520 IgG1/LALA recipients:  $p = 0.0298$  (Mantel-Cox test).

cells at low doses (Figures 3E and S4C). Groups of BALB/c laboratory mice were inoculated with 1,000 PFU of mouse-adapted EBOV, strain Mayinga (Bray et al., 1998), and 24 hr later treated by the intraperitoneal route with a low dose (20  $\mu\text{g}$  per mouse) of wild-type EBOV-520 IgG1 or EBOV-520 IgG1/LALA. EBOV-520 IgG1 did not mediate protection at this low dose, while 60% of mice treated with EBOV-520 IgG1/LALA survived (Figure 5). Since the disruption of Fc-Fc $\gamma$ R interactions by introduction of the LALA mutations is expected to reduce the ability of EBOV-520 to enhance infection, the protective effect observed after low-dose EBOV-520 IgG1/LALA is associated with disabling the enhancement potential *in vivo* that occurs when the mAb is present only in low concentration.

## DISCUSSION

ADE caused by EBOV GP-specific mAbs was first described by Takada et al. in 2001 using murine mAbs (Takada et al., 2001). It was hypothesized that ADE depends on epitope specificity of

mAbs (Nakayama et al., 2011; Takada et al., 2001, 2007). However, we found that ADE with human mAbs does not depend on the specific region of GP targeted by mAbs, similarly to ADE observed during dengue infection (Morens et al., 1987). Indeed, we observed ADE of EBOV and EBOV/BDBV-GP infections mediated by mAbs that recognize the glycan cap, the internal fusion loop, or the MPER (Figures 1B, S1, and S3A–S3D). Some mAbs targeting the EBOV GP glycan cap were found to cause ADE even at a high concentration of 100  $\mu\text{g}/\text{mL}$  and an MOI of 1.0 PFU/cell (BDBV41, BDBV52, BDBV43, EBOV87, 13C6), whereas others (BDBV270, BDBV289) with a similar epitope specificity did not. Induction of ADE may depend on fine differences in epitope specificity or differing binding poses of mAbs on similar epitopes. The MPER-specific mAb BDBV317 caused ADE at a much greater concentration than another MPER-specific mAb BDBV223 (Figure 1A). Moreover, EBOV-68 and EBOV-91, which bind to the C-terminal region of GP but do not neutralize EBOV (Figure S3A), increased the infection by 4- to 6-fold at  $\sim 100 \mu\text{g}/\text{mL}$  (Figure S3B). These data

suggest that each mAb has its own optimal concentration for neutralization, whereas at a lesser non-neutralizing concentration, it can cause ADE. Non-neutralizing mAbs appear to have the capacity to cause ADE even at high concentrations.

Antibodies recognize virus and engage Fc receptors on phagocytes, causing them to internalize virus-antibody complexes by Fc $\gamma$ R-mediated phagocytosis. If virus is not neutralized, this enhanced entry mechanism may mediate ADE (Chan et al., 2015; Flipse et al., 2013). However, ADE is not always observed in these settings, perhaps due to the destruction of pathogens in lysosomes or for other reasons (Halstead, 1994). In experiments with flaviviruses, formation of large antibody-virus aggregates with multiple viral particles was observed; moreover, the aggregates appeared to be taken up by macrophages (Gollins and Porterfield, 1985). Our experiments also demonstrated aggregation of viral particles in monocytic cells (Figure 2), which also could result in their increased uptake.

Engagement of Fc $\gamma$  receptors leads to reorganization of cytoskeleton and membrane remodeling resulting in enhancement of viral entry (Tay et al., 2016). Accordingly, disruption of the cytoplasmic tail of Fc $\gamma$ RI and Fc $\gamma$ RIIa reportedly abolished ADE (Furuyama et al., 2016; Rodrigo et al., 2006). Fc $\gamma$ RIII receptors were demonstrated to promote ADE via transmission of a phagocytic signal after activation (Park et al., 1993). As expected, we did not observe ADE in this study in the absence of Fc-mediated viral uptake (Figures 3A and S4A), suggesting that elimination of ADE by modification of Fc regions may be an attractive way to improve potency of mAbs (Williams et al., 2013).

Immunoglobulin subclass Fc domains have varying affinities to Fc $\gamma$  receptors (Hogarth and Pietersz, 2012), and consequently they mediate varying levels of effector function and phagocytic activity. The affinity of human immunoglobulins for binding to Fc $\gamma$  receptors in immune complexes formed by antibody and viral antigens may differ from that of free immunoglobulins. Immune complexes formed with human IgG1, IgG3, and IgG4 bind preferentially to Fc $\gamma$ RI, Fc $\gamma$ RIIa, and Fc $\gamma$ RIIIa, while those formed by IgG2 bind preferentially to Fc $\gamma$ RIIa and Fc $\gamma$ RIIIa (Bruhns et al., 2009; Liu, 2015; Tay et al., 2016). In our study, blocking of different Fc $\gamma$ Rs had differing blocking effects on the occurrence of ADE, which also differed for IgG1 and IgG3 mAbs: Fc $\gamma$ RI > Fc $\gamma$ RII > Fc $\gamma$ RIII, and Fc $\gamma$ RIII > Fc $\gamma$ RI > Fc $\gamma$ RII, respectively (Figures 3C and 3D). These data are consistent with different binding affinities of antibody subclasses to each type of Fc $\gamma$  receptor. Blocking of Fc $\gamma$ RI binding effectively abrogated ADE caused by the highly interacting subclass IgG1 (mAbs BDBV41, BDBV43, BDBV52) (Figure 3D) and less by subclass IgG3 antibodies (mAb BDBV223) (Figure 3C). However, blocking of Fc $\gamma$ RIII led to elimination of ADE mediated by IgG3 but not IgG1 antibodies (Figure 3D). A possible explanation for these data is the low expression of Fc $\gamma$ RIII receptors in THP-1 cells and high expression of Fc $\gamma$ RI (Tay et al., 2016). Similarly, change of IgG1 to IgG2 or IgG4 subclasses reduced ADE of dengue virus infection in THP-1 cells (Ramadhany et al., 2015), which bear Fc $\gamma$ RI and Fc $\gamma$ RII; the affinity of interactions of Fc $\gamma$ RII with IgGs is much lower (Swisher and Feldman, 2015). Interestingly, in K562 monocytic cells bearing only Fc $\gamma$ RII, the greatest level of ADE in experiments with dengue virus was induced by the

IgG2 subclass of D23-11G7C2 mAb (Ramadhany et al., 2015). Consistent with that finding, ADE of EBOV infection in K562 cells also was documented for IgG2 murine mAbs (Takada et al., 2007). These data suggest that induction of ADE depends greatly on the affinity between an antibody subclass and the specific Fc $\gamma$ R.

The LALA mutation of IgG1 mAbs BDBV41 and BDBV43 only slightly decreased ADE (Figure 3A). The LALA mutation initially was suggested to completely abolish binding to Fc $\gamma$ RI (Hezareh et al., 2001) but later was shown to reduce binding to Fc $\gamma$ RIIIa and Fc $\gamma$ RIIIa but not Fc $\gamma$ RI (Hessell et al., 2007); the effects were related to change in Fc glycosylation (Reusch and Tejada, 2015). These data are consistent with preferential binding of IgG1 to Fc $\gamma$ RI and the results of blocking Fc $\gamma$ Rs on induction of ADE by IgG1 (Figure 3B). Similarly, the KA mutation did not affect the level of ADE (Figure 3A). This mutation disables antibody-mediated complement activation (Thommesen et al., 2000) but only slightly reduces the binding affinity to Fc $\gamma$ RIIIa or Fc $\gamma$ RIIIa and does not affect binding to Fc $\gamma$ RI (Hezareh et al., 2001).

Our initial experiments were performed with two monocytic cell lines, which both demonstrated a low susceptibility to EBOV, consistent with previously published data (Martinez et al., 2013). In contrast, primary monocytes are highly permissive for EBOV infection (Ströher et al., 2001). The low susceptibility of monocytic cell lines to infection may be explained by their deficiency in  $\beta$ 1 integrins, which serve as an attachment factor of monocytes for EBOV (Dube et al., 2008). In addition, differentiation of THP-1 cells and expression of interferon-inducible transmembrane proteins promotes EBOV entry (Martinez et al., 2013). We observed a significant difference in virus susceptibility of the two human monocytic cell lines tested: 48 hr after infection with EBOV/BDBV-GP at 1.0 PFU/cell, the percentages of infected U937 cells varied from 0.5% to 1.3% (Figure 1C), but THP-1 cells varied from 3% to 8% (Figure 1C). Importantly, cells with lower susceptibility demonstrated higher levels of ADE: addition of BDBV41 resulted in a 50-fold increase of infected U937 cells but only a 6-fold increase of infected THP-1 cells (Figure 1C).

In humans, Fc $\gamma$ Rs have been detected on T and B lymphocytes, natural killer (NK) cells, neutrophils, granulocytes, monocytes, macrophages, and DCs (Nimmerjahn and Ravetch, 2008). To validate the cell line results above in primary human immune cells, we used total PBMCs, monocytes, immature, and mature DCs or macrophages obtained from human donors. Remarkable similarities have been identified between EBOV and dengue ADE in primary human myeloid cells (Halstead and O'Rourke, 1977; Halstead et al., 1977). Testing of filovirus infection in PBMCs demonstrated their very low susceptibility to infection, while that of primary monocytes, immature and mature DCs, and macrophages was markedly higher (Figure 4). Of interest, it has also been concluded that *in vitro*, dengue virus replicates in immature monocytes (Halstead et al., 1977). Moreover, dengue virus has been recovered from monocytes circulating in patients with acute dengue infection (Durbine et al., 2008), but this phenomenon is probably an adjunct to the predominant replication of virus in tissue macrophages (Aye et al., 2014). We found that EBOV-infected PBMCs transferred the virus to Vero E6 cells (Figure S5), suggesting that ADE may promote spread of filovirus

infection through various tissues. In the case of dengue ADE, an 8.5-fold increase of the proportion of infected primary human monocytes was accompanied with a 60-fold increase in the total virus output (Kou et al., 2011).

As was observed earlier during dengue infection (Boonnak et al., 2011), we found a very high donor-to-donor variability in susceptibility of primary immune cells to infection and ADE, which may be explained by differing levels of various cell surface molecules among samples. For example, primary human monocytes exhibit an extreme variability in expression of the inhibitory Fc $\gamma$ R1b, ranging from 1% to 48% (Boruchov et al., 2005). It is known that Fc $\gamma$ R1b limits phagocytosis (Liu et al., 2006), which is important for ADE. The low susceptibility of PBMC to EBOV infection may be explained by their low level of differentiation. The high donor-to-donor variability in susceptibility of primary monocytes to EBOV in our experiments (Figure 4) likely is explained by the extreme variability of expression of the inhibitory Fc $\gamma$ R1b in primary human monocytes (Boruchov et al., 2005) and the limitation of phagocytosis by Fc $\gamma$ R1b (Liu et al., 2006). Similarly, the extreme donor-to-donor variability in the levels of Fc $\gamma$ Rs and filovirus attachment factors may play a role in the varying levels of infection and ADE observed in the other primary immune cells tested.

As these data demonstrate that ADE can be caused by antibodies of different specificity and different subclasses, the ability of filovirus antibodies to cause ADE may be a consequence of some idiosyncratic features of filovirus particles, rather than antibodies themselves. For example, the unusual length of filovirus particles, typically around 1  $\mu$ M (Bharat et al., 2012), requires a large number of antibodies to saturate all GP spikes. It is possible that an incomplete saturation of antibodies on surface GP molecules results in ADE rather than neutralization. Finally, we demonstrated that ADE caused by a human EBOV-520 mAb *in vitro* is abrogated by mutagenesis of Fc domain (Figures 3E and S4C), and Fc-mediated effects prevent effective protection mediated by the same mAb *in vivo* (Figure 5).

These data have important implications for treatment of filovirus infections with passively transferred antibodies and for vaccinations. Low, sub-neutralizing concentrations of antibodies may cause ADE, suggesting that only very high concentrations of mAbs should be used for treatment of filovirus infections. Multiple failures of the early attempts to treat filovirus infections by passively transferred antibodies might be explained by insufficiently high doses of antibodies administered (reviewed in Kuhn, 2008). In contrast, the more recent successful experimental antibody treatments of filovirus infections used extremely high concentrations of monoclonal (Corti et al., 2016; Qiu et al., 2012, 2014) or polyclonal (Dye et al., 2012; Kudoyarova-Zubavichene et al., 1999; Pyankov et al., 2017) antibodies, which are likely to overcome the ADE effect. In this context, introduction of mutations in Fc domains that disable the interaction with Fc $\gamma$  receptors may be desirable. However, the Fc-Fc $\gamma$ R interaction also may contribute to antibody-mediated protection through Fc-mediated activation of phagocytic immune cells that contribute virus clearance and through activation of complement. Therefore, the complexity of interaction of therapeutic antibodies with immune cells should be considered carefully. Similarly, the success over the past decade in development of

filovirus vaccines is mostly associated with vaccine vectors able to replicate at very high levels, such as vesicular stomatitis virus, and/or administration of very high vaccine doses (Henao-Restrepo et al., 2015; Martins et al., 2016). As antibody levels in vaccinees wane over time, low concentrations of antibodies might contribute to ADE during subsequent filovirus exposure. This possibility should be carefully explored in experimentally vaccinated animals.

This study resulted in several important conclusions. First, filovirus GP-specific antibodies from human survivors present at low concentrations are capable of inducing ADE *in vitro*, suggesting that low levels of antibodies early after filovirus infections in humans may facilitate virus spread. Second, ADE can be caused by antibodies of various epitope specificities, neutralizing capacities and subclasses. Third, the ability of primary human immune cells to participate in ADE of infection varies greatly between cell types and is most pronounced in monocytes. Fourth, mutating the Fc domain to disable Fc-Fc $\gamma$ R interactions can enhance protection of a neutralizing antibody at low concentrations *in vivo*; hence, modification of the Fc region may increase the therapeutic efficacy of antibody treatments for EBOV-infected patients. Importantly, the data suggest that the dose of therapeutic mAbs injected to filovirus patients must be sufficiently high to achieve virus neutralizing concentrations *in vivo*, as lower doses may cause an opposite effect and enhance the infection.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
  - Viruses and cell lines
  - Isolation and culture of primary human immune cells
  - *In vivo* experiments
- METHOD DETAILS
  - Preparation of human mAbs, their mutated versions and Fab fragments
  - mAb-mediated enhancement of immune cells infection by filoviruses
  - Spread of infection from PBMCs to Vero E6 cells
  - Flow cytometry
  - Selection of EBOV/BDBV-GP escape mutants
  - Selection of VSV/BDBV-GP escape mutants
  - Confocal microscopy
- QUANTIFICATION AND STATISTICAL ANALYSIS

## SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and one table and can be found with this article online at <https://doi.org/10.1016/j.celrep.2018.07.035>.

## ACKNOWLEDGMENTS

We acknowledge the excellent technical support of Ms. Xiaoli Shen. This project received support from the U.S. NIH grant U19 AI109711 (to J.E.C. and A.B.)

and Defense Threat Reduction Agency grant HDTRA1-13-1-0034 (to J.E.C. and A.B.). We are grateful to Dr. Larry Zeitlin for providing plant-derived antibody BDBV43.

#### AUTHOR CONTRIBUTIONS

N.A.K., P.Y., P.G., R.I.S., A.I.F., P.A.I., K.H., N.M.L., and P.R. designed and performed the experiments as well as analyzed the data. N.A.K., R.I.S., P.A.I., J.E.C., and A.B. conceived the study, designed the experiments, analyzed the data, and wrote the manuscript. All authors commented on the manuscript.

#### DECLARATION OF INTERESTS

J.E.C. is a consultant for Sanofi and is on the Scientific Advisory Boards of PaxVax, CompuVax, GigaGen, and Meissa Vaccines, is a recipient of previous unrelated research grants from Moderna and Sanofi, and is founder of IDBiologics. P.A.I., P.G., A.B., and J.E.C. have applied for a patent that is related to this work. All other authors declared no competing interests.

Received: September 11, 2017

Revised: June 12, 2018

Accepted: July 10, 2018

Published: August 14, 2018

#### REFERENCES

- Acosta, E.G., and Bartenschlager, R. (2016). Paradoxical role of antibodies in dengue virus infections: Considerations for prophylactic vaccine development. *Expert Rev. Vaccines* *15*, 467–482.
- Ana-Sosa-Batiz, F., Johnston, A.P., Liu, H., Center, R.J., Rerks-Ngarm, S., Pit-suttithum, P., Nitayaphan, S., Kaewkungwal, J., Kim, J.H., Michael, N.L., et al. (2014). HIV-specific antibody-dependent phagocytosis matures during HIV infection. *Immunol. Cell Biol.* *92*, 679–687.
- Ana-Sosa-Batiz, F., Vanderven, H., Jegaskanda, S., Johnston, A., Rockman, S., Laurie, K., Barr, I., Reading, P., Lichtfuss, M., and Kent, S.J. (2016). Influenza-specific antibody-dependent phagocytosis. *PLoS ONE* *11*, e0154461.
- Aye, K.S., Charnkaew, K., Win, N., Wai, K.Z., Moe, K., Punyadee, N., Thiemmecca, S., Suttiheptumrong, A., Sukpanichnant, S., Prida, M., and Halstead, S.B. (2014). Pathologic highlights of dengue hemorrhagic fever in 13 autopsy cases from Myanmar. *Hum. Pathol.* *45*, 1221–1233.
- Baize, S., Leroy, E.M., Georges-Courbot, M.C., Capron, M., Lansoud-Soukate, J., Debré, P., Fisher-Hoch, S.P., McCormick, J.B., and Georges, A.J. (1999). Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. *Nat. Med.* *5*, 423–426.
- Bharat, T.A., Noda, T., Riches, J.D., Kraehling, V., Kolesnikova, L., Becker, S., Kawaoka, Y., and Briggs, J.A. (2012). Structural dissection of Ebola virus and its assembly determinants using cryo-electron tomography. *Proc. Natl. Acad. Sci. USA* *109*, 4275–4280.
- Boonnak, K., Dambach, K.M., Donofrio, G.C., Tassaneethitip, B., and Marovich, M.A. (2011). Cell type specificity and host genetic polymorphisms influence antibody-dependent enhancement of dengue virus infection. *J. Virol.* *85*, 1671–1683.
- Bornholdt, Z.A., Turner, H.L., Murin, C.D., Li, W., Sok, D., Souders, C.A., Piper, A.E., Goff, A., Shamblyn, J.D., Wollen, S.E., et al. (2016). Isolation of potent neutralizing antibodies from a survivor of the 2014 Ebola virus outbreak. *Science* *351*, 1078–1083.
- Borrok, M.J., Jung, S.T., Kang, T.H., Monzingo, A.F., and Georgiou, G. (2012). Revisiting the role of glycosylation in the structure of human IgG Fc. *ACS Chem. Biol.* *7*, 1596–1602.
- Boruchov, A.M., Heller, G., Veri, M.C., Bonvini, E., Ravetch, J.V., and Young, J.W. (2005). Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. *J. Clin. Invest.* *115*, 2914–2923.
- Bournazos, S., and Ravetch, J.V. (2017). Fc $\gamma$  receptor function and the design of vaccination strategies. *Immunity* *47*, 224–233.
- Bray, M., Davis, K., Geisbert, T., Schmaljohn, C., and Huggins, J. (1998). A mouse model for evaluation of prophylaxis and therapy of Ebola hemorrhagic fever. *J. Infect. Dis.* *178*, 651–661.
- Bruhns, P., Iannascoli, B., England, P., Mancardi, D.A., Fernandez, N., Jorieux, S., and Daéron, M. (2009). Specificity and affinity of human Fc $\gamma$  receptors and their polymorphic variants for human IgG subclasses. *Blood* *113*, 3716–3725.
- Burk, R., Bollinger, L., Johnson, J.C., Wada, J., Radoshitzky, S.R., Palacios, G., Bavari, S., Jahrling, P.B., and Kuhn, J.H. (2016). Neglected filoviruses. *FEMS Microbiol. Rev.* *40*, 494–519.
- Chan, K.R., Ong, E.Z., Mok, D.Z., and Ooi, E.E. (2015). Fc receptors and their influence on efficacy of therapeutic antibodies for treatment of viral diseases. *Expert Rev. Anti Infect. Ther.* *13*, 1351–1360.
- Clapham, H.E., Quyen, T.H., Kien, D.T., Dorigatti, I., Simmons, C.P., and Ferguson, N.M. (2016). Modelling virus and antibody dynamics during dengue virus infection suggests a role for antibody in virus clearance. *PLoS Comput. Biol.* *12*, e1004951.
- Corti, D., Misasi, J., Mulangu, S., Stanley, D.A., Kanekiyo, M., Wollen, S., Plouquin, A., Doria-Rose, N.A., Staupe, R.P., Bailey, M., et al. (2016). Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody. *Science* *351*, 1339–1342.
- Davidson, E., Bryan, C., Fong, R.H., Barnes, T., Pfaff, J.M., Mabila, M., Rucker, J.B., and Doranz, B.J. (2015). Mechanism of binding to ebola virus glycoprotein by the ZMapp, ZMAb, and MB-003 cocktail antibodies. *J. Virol.* *89*, 10982–10992.
- Dube, D., Schornberg, K.L., Stantchev, T.S., Bonaparte, M.I., Delos, S.E., Bouton, A.H., Broder, C.C., and White, J.M. (2008). Cell adhesion promotes Ebola virus envelope glycoprotein-mediated binding and infection. *J. Virol.* *82*, 7238–7242.
- Durbin, A.P., Vargas, M.J., Wanionek, K., Hammond, S.N., Gordon, A., Rocha, C., Balmaseda, A., and Harris, E. (2008). Phenotyping of peripheral blood mononuclear cells during acute dengue illness demonstrates infection and increased activation of monocytes in severe cases compared to classic dengue fever. *Virology* *376*, 429–435.
- Dye, J.M., Herbert, A.S., Kuehne, A.I., Barth, J.F., Muhammad, M.A., Zak, S.E., Ortiz, R.A., Prugar, L.I., and Pratt, W.D. (2012). Postexposure antibody prophylaxis protects nonhuman primates from filovirus disease. *Proc. Natl. Acad. Sci. USA* *109*, 5034–5039.
- Fibriansah, G., Ibarra, K.D., Ng, T.S., Smith, S.A., Tan, J.L., Lim, X.N., Ooi, J.S., Kostyuchenko, V.A., Wang, J., de Silva, A.M., et al. (2015). DENGUE VIRUS. Cryo-EM structure of an antibody that neutralizes dengue virus type 2 by locking E protein dimers. *Science* *349*, 88–91.
- Fleit, H.B., and Kobasiuk, C.D. (1991). The human monocyte-like cell line THP-1 expresses Fc gamma RI and Fc gamma RII. *J. Leukoc. Biol.* *49*, 556–565.
- Flipse, J., Wilschut, J., and Smit, J.M. (2013). Molecular mechanisms involved in antibody-dependent enhancement of dengue virus infection in humans. *Traffic* *14*, 25–35.
- Flyak, A.I., Ilinykh, P.A., Murin, C.D., Garron, T., Shen, X., Fusco, M.L., Hashiguchi, T., Bornholdt, Z.A., Slaughter, J.C., Sapparapu, G., et al. (2015). Mechanism of human antibody-mediated neutralization of Marburg virus. *Cell* *160*, 893–903.
- Flyak, A.I., Shen, X., Murin, C.D., Turner, H.L., David, J.A., Fusco, M.L., Lampley, R., Kose, N., Ilinykh, P.A., Kuzmina, N., et al. (2016). Cross-reactive and potent neutralizing antibody responses in human survivors of natural ebolavirus infection. *Cell* *164*, 392–405.
- Flyak, A.I., Kuzmina, N., Murin, C.D., Bryan, C., Davidson, E., Gilchuk, P., Gulka, C.P., Ilinykh, P.A., Shen, X., Huang, K., et al. (2018). Broadly neutralizing antibodies from human survivors target a conserved site in the Ebola virus glycoprotein HR2-MPER region. *Nat. Microbiol.* *3*, 670–677.

- Furuyama, W., Marzi, A., Carmody, A.B., Maruyama, J., Kuroda, M., Miyamoto, H., Nanbo, A., Manzoor, R., Yoshida, R., Igarashi, M., et al. (2016). Fc $\gamma$ -receptor IIa-mediated Src signaling pathway is essential for the antibody-dependent enhancement of ebola virus infection. *PLoS Pathog.* *12*, e1006139.
- Geisbert, T.W., Hensley, L.E., Larsen, T., Young, H.A., Reed, D.S., Geisbert, J.B., Scott, D.P., Kagan, E., Jahrling, P.B., and Davis, K.J. (2003). Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques: Evidence that dendritic cells are early and sustained targets of infection. *Am. J. Pathol.* *163*, 2347–2370.
- Gillis, C., Gouel-Chéron, A., Jönsson, F., and Bruhns, P. (2014). Contribution of Human Fc $\gamma$ Rs to Disease with Evidence from Human Polymorphisms and Transgenic Animal Studies. *Front. Immunol.* *5*, 254.
- Gollins, S.W., and Porterfield, J.S. (1985). Flavivirus infection enhancement in macrophages: An electron microscopic study of viral cellular entry. *J. Gen. Virol.* *66*, 1969–1982.
- Halstead, S.B. (1994). Antibody-dependent enhancement of infection: A mechanism for indirect virus entry into cells. *Cold Spring Harbor Monograph Archive 28: Cellular Receptors for Animal Viruses* (Cold Spring Harbor Laboratory Press), pp. 493–516.
- Halstead, S.B., and O'Rourke, E.J. (1977). Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J. Exp. Med.* *146*, 201–217.
- Halstead, S.B., O'Rourke, E.J., and Allison, A.C. (1977). Dengue viruses and mononuclear phagocytes. II. Identity of blood and tissue leukocytes supporting in vitro infection. *J. Exp. Med.* *146*, 218–229.
- Henao-Restrepo, A.M., Longini, I.M., Egger, M., Dean, N.E., Edmunds, W.J., Camacho, A., Carroll, M.W., Doumbia, M., Draguez, B., Duraffour, S., et al. (2015). Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: Interim results from the Guinea ring vaccination cluster-randomised trial. *Lancet* *386*, 857–866.
- Hessell, A.J., Hangartner, L., Hunter, M., Havenith, C.E., Beurskens, F.J., Bakker, J.M., Lanigan, C.M., Landucci, G., Forthal, D.N., Parren, P.W., et al. (2007). Fc receptor but not complement binding is important in antibody protection against HIV. *Nature* *449*, 101–104.
- Hezareh, M., Hessell, A.J., Jensen, R.C., van de Winkel, J.G., and Parren, P.W. (2001). Effector function activities of a panel of mutants of a broadly neutralizing antibody against human immunodeficiency virus type 1. *J. Virol.* *75*, 12161–12168.
- Higel, F., Seidl, A., Sörgel, F., and Friess, W. (2016). N-glycosylation heterogeneity and the influence on structure, function and pharmacokinetics of monoclonal antibodies and Fc fusion proteins. *Eur. J. Pharm. Biopharm.* *100*, 94–100.
- Hogarth, P.M., and Pietersz, G.A. (2012). Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond. *Nat. Rev. Drug Discov.* *11*, 311–331.
- Ilinykh, P.A., Shen, X., Flyak, A.I., Kuzmina, N., Ksiazek, T.G., Crowe, J.E., Jr., and Bukreyev, A. (2016). Chimeric filoviruses for identification and characterization of monoclonal antibodies. *J. Virol.* *90*, 3890–3901.
- Jahrling, P.B., Geisbert, J.B., Swarengen, J.R., Larsen, T., and Geisbert, T.W. (2007). Ebola hemorrhagic fever: Evaluation of passive immunotherapy in nonhuman primates. *J. Infect. Dis.* *196* (Suppl 2), S400–S403.
- Kou, Z., Lim, J.Y., Beltramello, M., Quinn, M., Chen, H., Liu, S., Martinez-Sobrido, L., Diamond, M.S., Schlesinger, J.J., de Silva, A., et al. (2011). Human antibodies against dengue enhance dengue viral infectivity without suppressing type I interferon secretion in primary human monocytes. *Virology* *410*, 240–247.
- Ksiazek, T.G., Rollin, P.E., Williams, A.J., Bressler, D.S., Martin, M.L., Swanepoel, R., Burt, F.J., Leman, P.A., Khan, A.S., Rowe, A.K., et al. (1999). Clinical virology of Ebola hemorrhagic fever (EHF): Virus, antigen, and IgM and IgG antibody findings among EHF patients in Kikwit, Democratic Republic of the Congo, 1995. *J. Infect. Dis.* *179* (Suppl 1), S177–S187.
- Kudoyarova-Zubavichene, N.M., Sergeev, N.N., Chepurnov, A.A., and Netesov, S.V. (1999). Preparation and use of hyperimmune serum for prophylaxis and therapy of Ebola virus infections. *J. Infect. Dis.* *179* (Suppl 1), S218–S223.
- Kuhn, J.H. (2008). *Filoviruses*, First Edition (Springer).
- Lee, J.E., Fusco, M.L., Hessell, A.J., Oswald, W.B., Burton, D.R., and Saphire, E.O. (2008). Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. *Nature* *454*, 177–182.
- Littau, R., Kurane, I., and Ennis, F.A. (1990). Human IgG Fc receptor II mediates antibody-dependent enhancement of dengue virus infection. *J. Immunol.* *144*, 3183–3186.
- Liu, L. (2015). Antibody glycosylation and its impact on the pharmacokinetics and pharmacodynamics of monoclonal antibodies and Fc-fusion proteins. *J. Pharm. Sci.* *104*, 1866–1884.
- Liu, Y., Gao, X., Masuda, E., Redecha, P.B., Blank, M.C., and Pricop, L. (2006). Regulated expression of Fc $\gamma$ RII in human dendritic cells controls cross-presentation of antigen-antibody complexes. *J. Immunol.* *177*, 8440–8447.
- Lubaki, N.M., Ilinykh, P., Pietzsch, C., Tigabu, B., Freiberg, A.N., Koup, R.A., and Bukreyev, A. (2013). The lack of maturation of Ebola virus-infected dendritic cells results from the cooperative effect of at least two viral domains. *J. Virol.* *87*, 7471–7485.
- Lubaki, N.M., Younan, P., Santos, R.I., Meyer, M., Iampietro, M., Koup, R.A., and Bukreyev, A. (2016). The Ebola interferon inhibiting domains attenuate and dysregulate cell-mediated immune responses. *PLoS Pathog.* *12*, e1006031.
- Martinez, O., Johnson, J.C., Honko, A., Yen, B., Shabman, R.S., Hensley, L.E., Olinger, G.G., and Basler, C.F. (2013). Ebola virus exploits a monocyte differentiation program to promote its entry. *J. Virol.* *87*, 3801–3814.
- Martins, K.A., Jahrling, P.B., Bavari, S., and Kuhn, J.H. (2016). Ebola virus disease candidate vaccines under evaluation in clinical trials. *Expert Rev. Vaccines* *15*, 1101–1112.
- McElroy, A.K., Akondy, R.S., Davis, C.W., Ellebedy, A.H., Mehta, A.K., Kraft, C.S., Lyon, G.M., Ribner, B.S., Varkey, J., Sidney, J., et al. (2015). Human Ebola virus infection results in substantial immune activation. *Proc. Natl. Acad. Sci. USA* *112*, 4719–4724.
- Messaoudi, I., Amarasinghe, G.K., and Basler, C.F. (2015). Filovirus pathogenesis and immune evasion: Insights from Ebola virus and Marburg virus. *Nat. Rev. Microbiol.* *13*, 663–676.
- Mire, C.E., Geisbert, J.B., Marzi, A., Agans, K.N., Feldmann, H., and Geisbert, T.W. (2013). Vesicular stomatitis virus-based vaccines protect nonhuman primates against Bundibugyo ebolavirus. *PLoS Negl. Trop. Dis.* *7*, e2600.
- Mire, C.E., Geisbert, J.B., Borisevich, V., Fenton, K.A., Agans, K.N., Flyak, A.I., Deer, D.J., Steinkellner, H., Bohorov, O., Bohorova, N., et al. (2017). Therapeutic treatment of Marburg and Ravn virus infection in nonhuman primates with a human monoclonal antibody. *Sci. Transl. Med.* *9*, eaai8711.
- Morens, D.M., Venkateshan, C.N., and Halstead, S.B. (1987). Dengue 4 virus monoclonal antibodies identify epitopes that mediate immune infection enhancement of dengue 2 viruses. *J. Gen. Virol.* *68*, 91–98.
- Murin, C.D., Fusco, M.L., Bornholdt, Z.A., Qiu, X., Olinger, G.G., Zeitlin, L., Kobinger, G.P., Ward, A.B., and Saphire, E.O. (2014). Structures of protective antibodies reveal sites of vulnerability on Ebola virus. *Proc. Natl. Acad. Sci. USA* *111*, 17182–17187.
- Nakayama, E., Tomabeche, D., Matsuno, K., Kishida, N., Yoshida, R., Feldmann, H., and Takada, A. (2011). Antibody-dependent enhancement of Marburg virus infection. *J. Infect. Dis.* *204* (Suppl 3), S978–S985.
- Nimmerjahn, F., and Ravetch, J.V. (2008). Fc $\gamma$  receptors as regulators of immune responses. *Nat. Rev. Immunol.* *8*, 34–47.
- Olinger, G.G., Jr., Pettitt, J., Kim, D., Working, C., Bohorov, O., Bratcher, B., Hiatt, E., Hume, S.D., Johnson, A.K., Morton, J., et al. (2012). Delayed treatment of Ebola virus infection with plant-derived monoclonal antibodies provides protection in rhesus macaques. *Proc. Natl. Acad. Sci. USA* *109*, 18030–18035.

- Oswald, W.B., Geisbert, T.W., Davis, K.J., Geisbert, J.B., Sullivan, N.J., Jahrling, P.B., Parren, P.W., and Burton, D.R. (2007). Neutralizing antibody fails to impact the course of Ebola virus infection in monkeys. *PLoS Pathog.* 3, e9.
- Park, J.G., Isaacs, R.E., Chien, P., and Schreiber, A.D. (1993). In the absence of other Fc receptors, Fc gamma RIIIA transmits a phagocytic signal that requires the cytoplasmic domain of its gamma subunit. *J. Clin. Invest.* 92, 1967–1973.
- Pyankov, O.V., Setoh, Y.X., Bodnev, S.A., Edmonds, J.H., Pyankova, O.G., Pyankov, S.A., Pali, G., Belford, S., Lu, L., La, M., et al. (2017). Successful post-exposure prophylaxis of Ebola infected non-human primates using Ebola glycoprotein-specific equine IgG. *Sci. Rep.* 7, 41537.
- Qiu, X., Alimonti, J.B., Melito, P.L., Fernando, L., Ströher, U., and Jones, S.M. (2011). Characterization of Zaire ebolavirus glycoprotein-specific monoclonal antibodies. *Clin. Immunol.* 141, 218–227.
- Qiu, X., Audet, J., Wong, G., Pillet, S., Bello, A., Cabral, T., Strong, J.E., Plummer, F., Corbett, C.R., Alimonti, J.B., and Kobinger, G.P. (2012). Successful treatment of ebola virus-infected cynomolgus macaques with monoclonal antibodies. *Sci. Transl. Med.* 4, 138ra81.
- Qiu, X., Wong, G., Audet, J., Bello, A., Fernando, L., Alimonti, J.B., Fausther-Bovendo, H., Wei, H., Aviles, J., Hiatt, E., et al. (2014). Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. *Nature* 514, 47–53.
- Qiu, X., Audet, J., Lv, M., He, S., Wong, G., Wei, H., Luo, L., Fernando, L., Kroeker, A., Fausther Bovendo, H., et al. (2016). Two-mAb cocktail protects macaques against the Makona variant of Ebola virus. *Sci. Transl. Med.* 8, 329ra33.
- Ramadhany, R., Hirai, I., Sasaki, T., Ono, K., Ramasoota, P., Ikuta, K., and Kurosu, T. (2015). Antibody with an engineered Fc region as a therapeutic agent against dengue virus infection. *Antiviral Res.* 124, 61–68.
- Reusch, D., and Tejada, M.L. (2015). Fc glycans of therapeutic antibodies as critical quality attributes. *Glycobiology* 25, 1325–1334.
- Rodrigo, W.W., Jin, X., Blackley, S.D., Rose, R.C., and Schlesinger, J.J. (2006). Differential enhancement of dengue virus immune complex infectivity mediated by signaling-competent and signaling-incompetent human Fc gamma RIA (CD64) or Fc gamma RIIIA (CD32). *J. Virol.* 80, 10128–10138.
- Sanchez, A., Trappier, S.G., Mahy, B.W., Peters, C.J., and Nichol, S.T. (1996). The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. *Proc. Natl. Acad. Sci. USA* 93, 3602–3607.
- Ströher, U., West, E., Bugany, H., Klenk, H.D., Schnittler, H.J., and Feldmann, H. (2001). Infection and activation of monocytes by Marburg and Ebola viruses. *J. Virol.* 75, 11025–11033.
- Swisher, J.F., and Feldman, G.M. (2015). The many faces of FcγRI: Implications for therapeutic antibody function. *Immunol. Rev.* 268, 160–174.
- Takada, A., Watanabe, S., Okazaki, K., Kida, H., and Kawaoka, Y. (2001). Infectivity-enhancing antibodies to Ebola virus glycoprotein. *J. Virol.* 75, 2324–2330.
- Takada, A., Feldmann, H., Ksiazek, T.G., and Kawaoka, Y. (2003). Antibody-dependent enhancement of Ebola virus infection. *J. Virol.* 77, 7539–7544.
- Takada, A., Ebihara, H., Feldmann, H., Geisbert, T.W., and Kawaoka, Y. (2007). Epitopes required for antibody-dependent enhancement of Ebola virus infection. *J. Infect. Dis.* 196 (Suppl 2), S347–S356.
- Tay, M.Z., Liu, P., Williams, L.D., McRaven, M.D., Sawant, S., Gurley, T.C., Xu, T.T., Dennison, S.M., Liao, H.X., Chenine, A.L., et al. (2016). Antibody-Mediated Internalization of Infectious HIV-1 Virions Differs among Antibody Isoforms and Subclasses. *PLoS Pathog.* 12, e1005817.
- Thommesen, J.E., Michaelsen, T.E., Løset, G.A., Sandlie, I., and Brekke, O.H. (2000). Lysine 322 in the human IgG3 C(H)2 domain is crucial for antibody dependent complement activation. *Mol. Immunol.* 37, 995–1004.
- Towner, J.S., Paragas, J., Dover, J.E., Gupta, M., Goldsmith, C.S., Huggins, J.W., and Nichol, S.T. (2005). Generation of eGFP expressing recombinant Zaire ebolavirus for analysis of early pathogenesis events and high-throughput antiviral drug screening. *Virology* 332, 20–27.
- Volchkov, V.E., Becker, S., Volchkova, V.A., Ternovoj, V.A., Kotov, A.N., Nete-sov, S.V., and Klenk, H.D. (1995). GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. *Virology* 214, 421–430.
- Williams, K.L., Sukupolvi-Petty, S., Beltramello, M., Johnson, S., Sallusto, F., Lanzavecchia, A., Diamond, M.S., and Harris, E. (2013). Therapeutic efficacy of antibodies lacking Fcγ receptor binding against lethal dengue virus infection is due to neutralizing potency and blocking of enhancing antibodies [corrected]. *PLoS Pathog.* 9, e1003157.
- Wilson, J.A., Hevey, M., Bakken, R., Guest, S., Bray, M., Schmaljohn, A.L., and Hart, M.K. (2000). Epitopes involved in antibody-mediated protection from Ebola virus. *Science* 287, 1664–1666.
- Zeitlin, L., Pettitt, J., Scully, C., Bohorova, N., Kim, D., Pauly, M., Hiatt, A., Ngo, L., Steinkellner, H., Whaley, K.J., and Olinger, G.G. (2011). Enhanced potency of a fucose-free monoclonal antibody being developed as an Ebola virus immunoprotectant. *Proc. Natl. Acad. Sci. USA* 108, 20690–20694.

## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Antibodies</b>		
13C6 (mouse-human chimeric, produced in CHO)	Wilson et al., 2000 Olinger et al., 2012	N/A
2G4 (mouse-human chimeric, produced in CHO)	Qiu et al., 2011 Qiu et al., 2016	N/A
2D22 (hybridoma-produced IgG1)	Fibriansah et al., 2015	N/A
BDBV43 (produced in plant)	Mapp Biopharmaceutical	N/A
F(ab') fragments, (from BDBV41 produced in CHO)	Flyak et al., 2016	N/A
F(ab') <sub>2</sub> fragments, (from BDBV41 produced in CHO)	Flyak et al., 2016	N/A
F(ab') <sub>2</sub> fragments, (from BDBV43 produced in CHO)	Flyak et al., 2016	N/A
BDBV52	Flyak et al., 2016	N/A
BDBV41	Flyak et al., 2016	N/A
BDBV43	Flyak et al., 2016	N/A
BDBV223	Flyak et al., 2016, 2018	N/A
BDBV270	Flyak et al., 2016	N/A
BDBV259	Flyak et al., 2016	N/A
BDBV289	Flyak et al., 2016	N/A
BDBV317	Flyak et al., 2016, 2018	N/A
BDBV324	Flyak et al., 2016	N/A
EBOV-520	P.G., N.K., P.A.I., K.H., A.I.F., A.B., J.E.C., B.M. Gunn, A. Bryan, E. Davidson, B.J. Doranz, H.L. Turner, M.L. Fusco, M.S. Bramble, N.A. Hoff, E. Binshtein, N. Kose, R. Flinko, C. Orlandi, R. Carnahan, E.H. Parrish, A.M. Sevy, R.G. Bombardi, P.K. Singh, P. Mukadi, J.J. Muyembe-Tamfum, M.D. Ohi, E. Ollmann Saphire, G.K. Lewis, G. Alter, A.B. Ward, and A.W. Rimon, unpublished data	N/A
EBOV-87	This paper	N/A
EBOV-141	This paper	N/A
EBOV-333	This paper	N/A
EBOV-91	This paper	N/A
EBOV-293	This paper	N/A
MR72	Flyak et al., 2015	N/A
MR114	Flyak et al., 2015	N/A
MR144	Flyak et al., 2015	N/A
MR191	Flyak et al., 2015	N/A
MR228	Flyak et al., 2015	N/A
CD64 Monoclonal Antibody (10.1)	ThermoFisher Scientific	Cat# MA1-10270
CD16 Monoclonal Antibody (3G8)	ThermoFisher Scientific	Cat# MA1-10112
Polyclonal Goat IgG Human Fc gamma RII/CD32 Antibody	R&D System	Cat# AF1330
Anti-human CD1c	BioLegend	Cat# 331502
Donkey Anti-human IgG Conjugated With CF594	Sigma-Aldridge	Cat# SAB4600097
Anti-BDBV GP Rabbit Polyclonal Antibodies	Integrated BioTherapeutics	Cat# 0304-001
Donkey anti-rabbit antibodies conjugated with AlexaFluor 647	Invitrogen	Cat# A-31573
Donkey anti-mouse antibodies conjugated with AlexaFluor 488	Invitrogen	Cat# A-21206

(Continued on next page)

<b>Continued</b>		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Bacterial and Virus Strains</b>		
Recombinant EBOV expressing eGFP	Towner et al., 2005	N/A
EBOV/BDBV-GP	Ilinykh et al., 2016	N/A
EBOV/SUDV-GP	Ilinykh et al., 2016	N/A
EBOV/MARV-GP	Ilinykh et al., 2016	N/A
Mouse-adapted EBOV strain Mayinga	Bray et al., 1998	N/A
VSV/BDBV-GP	Geisbert Lab., UTMB	N/A
<b>Biological Samples</b>		
Buffy coats	UTMB Blood Bank	N/A
<b>Chemicals, Peptides, and Recombinant Proteins</b>		
MEM medium	ThermoFisher Scientific	Cat# 11095-098
HyClone Fetal Bovine Serum	ThermoFisher Scientific	Cat# SH3007003
RPMI 1640 medium	Invitrogen	Cat# 11875-119
Histopaque	Sigma-Aldrich	Cat# 10771-100ML
Human Granulocyte-macrophage Colony-stimulating factor	Peptotech	Cat# 300-03
HiTrap Protein G	Sigma-Aldrich	Cat# GE29-0485-81
MoDC maturation medium	Miltenyi Biotec	Cat# 130-094-813
TRIzol Reagent	Thermo Fisher Scientific	Cat# 15596026
Phosphate-buffered formalin	ThermoFisher Scientific	Cat# SF100-4
PBS	GIBCO	Cat# 14190
Triton X-100	Thermo Fisher Scientific	Cat# 28314
Bovine serum albumin	Sigma-Aldrich	Cat# A8531-1VL
DAPI	Invitrogen	Cat# D1306
PermaFluor mounting medium	ThermoFisher Scientific	Cat# TA-030-FM
Paraformaldehyde	Electron Microscopy Sciences	Cat# 30525-89-4
Recombinant Human IL-4	Peptotech	Cat# 200-04
<b>Critical Commercial Assays</b>		
CD14 Microbeads, human	Miltenyi Biotec	Cat# 130-042-401
<b>Experimental Models: Cell Lines</b>		
U937	ATCC	Cat# CRL-1593.2
THP-1	ATCC	Cat# TIB-202
Vero-E6	ATCC	Cat# CRL-1586
Human primary cell isolates	This paper	N/A
Human primary cell culture	This paper	N/A
<b>Experimental Models: Organisms/Strains</b>		
BALB/c laboratory mice	Charles River Laboratories	Strain code: 028
<b>Software and Algorithms</b>		
PRISM	GraphPad Software	Version 6.05
FlowJo	Tree Star	Version 10.0.8
C6 analysis software for PC	BD Biosciences	Version 1.0.264.21
<b>Other</b>		
Accuri C6	BD Biosciences	N/A
LSRII Fortessa cytometer	BD Biosciences	N/A

## CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Alexander Bukreyev ([alexander.bukreyev@utmb.edu](mailto:alexander.bukreyev@utmb.edu)).

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

### Viruses and cell lines

The following filoviruses were used in the study: the recombinant EBOV expressing eGFP from an added transcriptional cassette (Towner et al., 2005), and its derivatives in which GP was replaced with its counterpart from BDBV (EBOV/BDBV-GP), SUDV (EBOV/SUDV-GP) and MARV (EBOV/MARV-GP) (Ilinykh et al., 2016), and mouse-adapted EBOV strain Mayinga (Bray et al., 1998). The viruses were propagated in Vero E6 cells and titrated in monolayers of Vero E6 cells (ATCC). Following a 1 hr-long adsorption, virus dilutions were covered with 0.4% methylcellulose overlay in MEM medium (ThermoFisher Scientific) containing 2% fetal bovine serum (HyClone). Plates were incubated at 37°C with 5% CO<sub>2</sub> for three days, and plaques were counted using a fluorescent microscope. The pro-monocytic human myeloid leukemia cell line U937 (ATCC) and the human monocytic leukemia cell line THP-1 (ATCC) were cultured in RPMI 1640 medium (ThermoFisher Scientific) supplemented with 10% fetal bovine serum (HyClone) at 37°C with 5% CO<sub>2</sub>. All work with filoviruses was performed in the BSL-4 facility of the Galveston National Laboratory.

### Isolation and culture of primary human immune cells

Blood samples were collected from anonymous healthy adult donors at the UTMB Blood Bank according to a clinical protocol approved by the UTMB Institutional Review Board. Buffy coats from blood samples were used for isolation of PBMCs by density gradient centrifugation in Ficoll (Histopaque; Sigma-Aldrich). CD14<sup>+</sup> monocytes were purified using anti-human CD14 antibody-labeled magnetic beads and magnetic LS columns (Miltenyi Biotec) and used immediately or further differentiated into DCs or macrophages. Isolated monocytes typically showed > 90% positivity for CD14 staining by flow cytometry. Monocytes were plated at  $0.7 \times 10^6$  to  $1 \times 10^6$  cells/mL in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum (HyClone) and either 500 U/mL of human granulocyte-macrophage colony-stimulating factor (GM-CSF; Peprotech) and 500 U/mL of human interleukin-4 (IL-4; Peprotech) to differentiate into immature DCs, or 20-50 ng/mL of macrophage colony-stimulating factor (M-CSF; Peprotech) to differentiate into macrophages and cultured for 7 days. Immature DCs expressed surface CD11c, CD1c, and HLA-DR and were low in CD14, whereas macrophages retained high levels of CD14 and were HLA-DR positive as determined by flow cytometry. To generate mature DCs, MoDC maturation (Miltenyi Biotec) medium was added to immature DCs, and cells were incubated for an additional 3 days.

### In vivo experiments

The animal protocol for testing of mAbs in laboratory mice was approved by the UTMB Institutional Animal Care and Use Committee. Seven-week-old BALB/c laboratory mice (Charles River Laboratories) were placed in the ABSL-4 facility of the Galveston National Laboratory. Groups of laboratory mice at 4-5 animals per group were injected intraperitoneally with 1,000 PFU of the mouse-adapted EBOV. Twenty-four hours later, animals were injected with 20 µg mAbs by the intraperitoneal route. Animals treated with 2D22 mAb served as controls. The animal observation procedure was performed as previously described (Flyak et al., 2016). The overall observation period lasted for 28 days.

## METHOD DETAILS

### Preparation of human mAbs, their mutated versions and Fab fragments

Human hybridoma cell lines were expanded in post-fusion medium, as previously described (Flyak et al., 2015). Supernatants from cultured hybridoma cells were collected, clarified by low-speed centrifugation and filtered. HiTrap Protein G or HiTrap MabSelectSure columns were used to purify antibodies from filtered supernatants. F(ab') fragments were generated by papain digestion, as described previously (Flyak et al., 2015) and F(ab')<sub>2</sub> fragments were produced by pepsin digestion. Generation and characterization of BDBV-specific mAbs was described previously (Flyak et al., 2016). Plant-derived BDBV43 was generated as previously described (Zeitlin et al., 2011). Genes encoding the immunoglobulins with variant Fc region were synthesized on a BioXP DNA synthesizer (Synthetic Genomics) and Gibson cloned into an antibody expression vector. Plasmid maxiprep DNAs were prepared in *E. coli* and purified using commercial DNA kits (QIAGEN). DNAs were used to transfect 293F cells (ThermoFisher Scientific) for recombinant antibody expression. IgGs were purified from transfected cell supernatants by chromatography using HiTrap Protein G or HiTrap MabSelectSure columns.

### mAb-mediated enhancement of immune cells infection by filoviruses

The monocytic cell lines (THP-1, U937) or primary human immune cells (PBMCs, monocytes, DCs or macrophages) were pre-incubated in 24-well plates at  $2 \times 10^5$  cells per well with mAbs at indicated concentrations for 1 hour, inoculated with EBOV, EBOV/BDBV-GP, EBOV/SUDV-GP or EBOV/MARV-GP viruses at an indicated MOI, and incubated at 37°C with 5% CO<sub>2</sub>. At 48 hours (or 0 or 24 hours where indicated) after inoculation, cells were harvested by low-speed centrifugation (200 x g, 10 min), washed once with PBS, fixed with 4% paraformaldehyde and analyzed by flow cytometry. In a separate experiment, THP-1 cells were pre-incubated with mAbs at 100 µg/mL for 1 hour, inoculated with EBOV/BDBV-GP at an MOI of 1 PFU/cell and incubated for 1 hour at 37°C, 5% CO<sub>2</sub>. Then, cells were washed 3 times with PBS, resuspended in RPMI 1640 medium with 10% fetal bovine serum

and incubated at 37°C with 5% CO<sub>2</sub>. At 48 hours after infection, cell suspensions were centrifuged at 200 x g for 5 min, and supernatants were harvested and titrated on Vero E6 monolayers as described above.

### Spread of infection from PBMCs to Vero E6 cells

Isolated PBMCs in 24-well plates at  $2 \times 10^5$  cells per well were inoculated with EBOV/BDBV-GP virus at an MOI of 1 PFU/cell, and incubated at 37°C with 5% CO<sub>2</sub>. At 24 hours after infection, cells were harvested by low-speed centrifugation (200 x g, 5 min), resuspended in 1 mL MEM medium with 10% fetal bovine serum and placed at the top of Vero E6 cell monolayers, and incubated for 48 hours at 37°C with 5% CO<sub>2</sub>. Next, monolayers were washed 3 times with PBS to remove PBMCs and residues of MEM medium. Vero E6 cells then were treated with trypsin, harvested, washed twice with PBS and fixed with 4% paraformaldehyde for the subsequent flow cytometry analysis.

### Flow cytometry

Following infection with recombinant filoviruses expressing eGFP, cells were fixed in 4% paraformaldehyde for 24 hours, followed by a second fixation in fresh paraformaldehyde, and then taken out of the BSL-4 facility according to the approved standard operation procedure. Then, fixed cells were centrifuged at 450 x g for 10 min, resuspended in PBS and analyzed by analytical flow cytometry using an Accuri C6 or LSRII Fortessa cytometer (both BD Biosciences) at the UTMB Flow Cytometry Core Facility. The acquired data were analyzed using FlowJo 7.6.1 software (Tree Star). The percentages of eGFP<sup>+</sup> cells in total cell populations were calculated.

### Selection of EBOV/BDBV-GP escape mutants

To select escape mutants, 100 PFU of EBOV/BDBV-GP were combined with 2-fold dilutions of mAbs starting at 200 μg/mL in u-bottom 96-well plates and incubated for 1 hour at 37°C. Mixtures were placed on Vero E6 cell monolayers in flat bottom 96-well plates and incubated for 1 hour. Supernatants were removed, fresh mAbs were added at the same concentrations in 200 μL of MEM supplemented with 2% FBS, and cells were incubated for 7 days at 37°C. Supernatants from wells in which viral replication was observed in the presence of the highest concentrations of mAbs, as determined by UV microscopy, were collected. 20 μL aliquots were incubated with 2-fold dilutions of mAbs starting at 200 μg/mL, and viruses were propagated in the presence of mAbs as above. The procedure was repeated once more with mAb dilutions starting at 400 μg/mL. Viruses that replicated at the highest mAb concentrations were amplified in Vero E6 cell culture monolayers in 24-well plates in the presence of mAbs at 200 μg/mL for 7 days. RNA was isolated from infected cells using TRIzol, and GP genes were RT-PCR-amplified and sequenced. To determine susceptibility of the isolated escape mutants to mAbs, 100 PFU of the viruses in MEM supplemented with 2% FBS in triplicate were combined in U-bottom 96-well plates with 8 to 12 two-fold dilutions of mAbs, starting at 200 μg/mL, in total volumes of 50 μL, and incubated for 1 hr at 37°C. The virus/antibody mixtures then were placed in triplicate Vero E6 cell culture monolayers in 96-well plates, incubated for 1 hr at 37°C, washed with MEM, overlaid with 200 μL of MEM containing 2% FBS and 0.8% methylcellulose, and incubated for 48 hours at 37°C. Plates were fixed with 10% phosphate-buffered formalin (ThermoFisher Scientific), taken out of the BSL-4 facility and plaques were counted using a fluorescence microscope.

### Selection of VSV/BDBV-GP escape mutants

The mutated chimeric vesicular stomatitis viruses covered with BDBV GP (VSV/BDBV-GP) able to resist neutralization by mAbs were selected as described earlier (Flyak et al., 2015). Briefly, 200 PFU of VSV/BDBV-GP (Mire et al., 2013) (provided by Dr. C. Mire and Dr. T. Geisbert, UTMB) were pre-neutralized before each passage by two-fold serial mAb dilutions starting from 200 μg/mL for 1 hour at 37°C passaged several times in Vero E6 cells in the presence of corresponding amount of mAb for 2 days. After each passage, harvested virus aliquots were titrated, and virus-positive sample with highest mAb concentrations were used for subsequent passage. Finally, virus plaques were grown under mAb-containing 0.7% agarose overlay for 5 days, visualized by neutral red staining, purified, and used for propagation of VSV/BDBV-GP escape mutants in presence of corresponding mAb. GP of propagated viruses were RT-PCR-amplified and sequenced, and mutants were assessed for their mAb resistance in a standard plaque reduction assay compared to the initial VSV/BDBV-GP virus as described above for EBOV/BDBV-GP.

### Confocal microscopy

THP-1 cells were grown in suspension, inoculated with EBOV/BDBV-GP at an MOI of 5 PFU/cell and fixed with 4% formaldehyde at room temperature for 15 min. Cells were rehydrated with PBS, permeabilized with 0.5% Triton X-100 in PBS for 15 min and washed three times with PBS to remove the permeabilization solution. Next, antigen was blocked with 5% donkey serum, 1% bovine serum albumin and 0.1% Triton X-100 (PBS-BSA-TX100) in PBS for 1 hour. For EBOV/BDBV-GP staining, rabbit immune serum against EBOV virus-like particles and anti-BDBV GP rabbit polyclonal antibodies (Integrated BioTherapeutics) were diluted at 1:100 each in PBS-BSA-TX100. For co-staining of FcRs, murine mAbs specific for FcγRIII (clone GRM1, Southern Biotech) were added to the anti-BDBV mixture at a 1:50 dilution, and then cells were incubated for one hour at room temperature and washed 5 times in PBS with 0.1% Triton X-100. Next, donkey anti-rabbit antibodies conjugated with AlexaFluor 647 (Invitrogen) and donkey anti-mouse antibodies conjugated with AlexaFluor 488 (Invitrogen) were added at a 1:200 dilution, and cells were incubated for 1 hour at room temperature. For human mAb staining, donkey anti-human IgG conjugated with CF594 (catalog #SAB4600097, Sigma-Aldridge) was added at 1:50 dilution and cells were incubated for 1 hour at room temperature. Slides were washed four times in PBS-BSA-TX100,

cells were fixed in 4% formaldehyde for 72 hours and removed from the BSL-4 facility. Next, cells were washed once with 0.5 M glycine diluted in PBS, incubated with 6-diamin-2-phenylindole-dihydrochloride (DAPI) (Invitrogen) at 1  $\mu\text{g}/\text{mL}$  for 2 min for nuclei staining, and washed five times with PBS. The slides were washed and mounted onto coverslips using PermaFluor mounting medium (ThermoFisher Scientific) and visualized under confocal microscope. Laser scanning confocal microscopy was performed using an Olympus FV1000 confocal microscope housed at the Galveston National Laboratory. All images were acquired using a 60x oil objective.

#### QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses and generations of graphs were performed using GraphPad Prism version 6.05 (GraphPad Software). Statistical significances were calculated using t test, multiple or unpaired. In the experiments with primary human immune cells (Figure 4), statistical significance was determined using the Holm-Sidak method, with  $\alpha = 5\%$ . For analysis of survival data in *in vivo* experiments (Figure 5), log-rank (Mantel-Cox) test was used.