Published in final edited form as:

*Cell Rep*. 2018 August 14; 24(7): 1802–1815.e5. doi:10.1016/j.celrep.2018.07.035.

# ANTIBODY-DEPENDENT ENHANCEMENT OF EBOLA VIRUS INFECTION BY HUMAN ANTIBODIES ISOLATED FROM SURVIVORS

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

[1]Department of Pathology, University of Texas Medical Branch, Galveston, TX, 77555, USA.

[2]Galveston National Laboratory, Galveston, TX, 77550, USA.

[3]Department of Microbiology & Immunology, University of Texas Medical Branch, Galveston, TX, 77555, USA.

[4]Department of Pathology, Microbiology, and Immunology, Vanderbilt University, Nashville, TN, 37232, USA.

[5]Vanderbilt Vaccine Center, Vanderbilt University Medical Center, Nashville, TN, 37232, USA.

[6]Department of Pediatrics, Vanderbilt University Medical Center, Nashville, TN, 37232, USA.

[7]Current address: Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, 91125, USA.

## 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 Fcγ 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 a mAb that altered its

*Address correspondence to: Alexander Bukreyev, alexander.bukreyev@utmb.edu or James E. Crowe, Jr., james.crowe@vanderbilt.edu.

AUTHOR CONTRIBUTIONS

NK, PY, PG, RIS, AIF, PAI, KH, NML and PR designed and performed the experiments as well as analyzed the data. NK, RIS, PAI, JEC and AB conceived the study, designed the experiments, analyzed the data and wrote the manuscript. All authors commented on the manuscript.

Lead Contact: Alexander Bukreyev, Departments of Pathology and Microbiology & Immunology, Galveston National Laboratory, Keiller Building, Room 3.145, University of Texas Medical Branch, 301 University Boulevard, Galveston, Texas 77555-0609, USA. Phone: 409-772-2829; alexander.bukreyev@utmb.edu.

DECLARATION OF IN ITERESTS

J.E.C. is a consultant for Sanofi, and is on the Scientific Advisory Boards of PaxVax, CompuVax, GigaGen, 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 a patent applied for that is related to this work. All other authors declared no competing interests.

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, IgM and IgG responses appeared in patients on days 8–10 after disease onset (Ksiazek et al., 1999). Survivors of the 1996 EBOV outbreak in Gabon had greater antibody responses than those that succumbed (Baize et al., 1999). A detailed characterization of the immune status of four EBOV 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 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 ~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 was 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; Flyak et al., 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 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 (Gilchuk et al., 2018) 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; Flyak et al., 2018; Flyak et al., 2016). To test if 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) (Fig. S1A, B). 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 green fluorescent protein (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 μg/mL and inoculated them at a multiplicity of infection (MOI) of 1.0, 0.1 or 0.01 PFU/cell (as determined by virus titration in Vero E6 cell monolayers). The cells were incubated for 48 hours, and the numbers of infected ($eGFP^+$) cells were quantified by flow cytometry (Fig. 1A, B). Strikingly, we detected a reduction in the number of $eGFP^+$ 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 (Fig. 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 (Fig. S1A) and have comparable 50% maximal inhibitory concentrations ($IC_{50}$). 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.

Since the percentage of $eGFP^+$ cells was used as a measure of ADE, it was important to confirm that mAb-mediated increase of $eGFP^+$ 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 Fig. 1B (BDBV52, BDBV41 and BDBV43), inoculated THP-1 cells with virus/mAb mixtures, incubated for 1 hour, washed cells with PBS, resuspended them in fresh medium with no mAbs added, and determined the virus titers in cell supernatants at 48 hours after infection (Fig. 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 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 $eGFP^+$

cells (Fig. 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 (Fig. 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 hours later. The delayed treatment also induced ADE, which was only marginally lower than that following treatment applied immediately after infection (Fig. 1E).

We next sought to determine if 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 $IC_{50}$ (Fig. 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 (Gilchuk et al., 2018), which binds in the region near the IFL but in an unusual binding pose that alters receptor binding 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 (Fig. 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; Qiu et al., 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 (Fig. S3A, B).

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 (Fig. 1), BDBV324 (Flyak et al., 2016), EBOV-293 (unpublished) and EBOV-520 (Gilchuk et al., 2018). 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 ($eGFP^+$) cells, except EBOV-520 which significantly reduced infection at this concentration (Fig. 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 (Fig. 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 ($eGFP^+$) 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.

## ADE depends on the presence of Fc fragments and Fcγ 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 FcγRs 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 one hour; we observed aggregation of viral particles and co-localization of aggregates with Fc receptors in presence of BDBV223 (Fig. 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 $F(ab')_2$ fragments of BDBV41 or BDBV43 did not cause ADE (Fig. 3A, 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 μg/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 (Fig. S4A, B). When cells were inoculated with EBOV, which is less sensitive to neutralization by BDBV223 (Fig. 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) (Fig. S4A, B), 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 Fcγ receptors, FcγRI, FcγRII or FcγRIII, on ADE. THP-1 cells were inoculated with FcR-blocking antibodies for 1 hour, treated with recombinant BDBV223 IgG1, IgG2, IgG3 or IgG4 mAbs at a concentration 0.1 μg/mL, and inoculated with EBOV/BDBV-GP at an MOI of 1.0 PFU/cell (Fig. 3B). As recombinant IgG1, IgG2 or IgG4 did not cause ADE, blocking of any of the receptors resulted in little or no effect. In contract, when IgG3 was used, blocking of FcγRIII eliminated ADE, while blocking of FcγRI or FcγRII significantly reduced it. Testing of hybridoma-produced BDBV223 antibody with EBOV, which is less sensitive to this mAb (Fig. S1B), also demonstrated the greatest reduction of ADE by blocking of FcγRIII and much lesser effects by blocking of FcγRI or FcγRII (Fig. 3C). These data are surprising, because a single THP-1 cell expresses FcγRI 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). Interestingly, unlike these data, dengue ADE is associated primarily with FcγRI and FcγRII (Acosta and Bartenschlager, 2016; Littaua 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 FcγRI, but not FcγRII or FcγRIII (Fig. 3D), which is consistent with the highest binding affinity of FcγRI 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 FcγRIIa and FcγRIIIa but not FcγRI (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 (Fig. 3A). These data are consistent with the preferential binding of IgG1 to FcγRI. The LALA mutation of EBOV-520 (IgG1 subclass as well) abrogated ADE *in vitro* even more efficiently (Fig. 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 (Fig. 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 FcγR 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 FcγRs 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* (Fig. 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 ADE of an infection in each of the cell types to a varying extent in cells obtained from many but not all donors. The PBMC samples revealed low susceptibility to infection, ranging from 0.2 to 6% of infected ($eGFP^+$) 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 $eGFP^+$ cells ranging from 7 to 37% between donors. The ADE observed in these cells was comparable in magnitude to that in PBMCs (Fig. 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 to 22%, or 3 to 21% infected cells, respectively, as in our previous study, Lubaki et al., 2013), which is somewhat lower than that observed for monocytes. 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 to 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 if 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 hours, 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 hours, then PBMCs were removed, Vero E6 monolayers washed with PBS, trypsinized, and the proportion of $eGFP^+$ Vero E6 cells was measured by flow cytometry. Vero E6 cells incubated with infected PBMCs from three donors showed the percentages of infected ($eGFP^+$) cells ranging from 45% to 67% (Fig. S5). This additional line of evidence shows that ADE caused by mAbs in innate 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 (Gilchuk et al., 2018). One hundred μg of the mAb uniformly protects laboratory mice against lethal EBOV infection when administered 24 h after challenge (Gilchuk et al., 2018). 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 FcγRs

(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 cells at low doses (Fig. 3E, 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 h later treated by the intraperitoneal route with a low dose (20 μg 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 (Fig. 5). Since the disruption of Fc-FcγR 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., 2007; Takada et al., 2001). 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 (Fig. 1B, S1, S3A–D). Some mAbs targeting the EBOV GP glycan cap were found to cause ADE even at a high concentration of 100 μg/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 (Fig. 1A). Moreover, EBOV-68 and EBOV-91, which bind to the C-terminal region of GP but do not neutralize EBOV (Fig. S3A), increased the infection by 4- to 6-fold at ~100 μg/mL (Fig. S3B). These data suggest that each mAb has its own optimal concentration for neutralization, whereas at a lesser non-neutralizing concentration they 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 FcγR-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 (Fig. 2), which also could result in their increased uptake.

Engagement of Fcγ 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 FcγRI and FcγRIIa reportedly abolished ADE (Furuyama et al., 2016; Rodrigo et al., 2006). FcγRIII 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 (Fig 3A, 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 Fcγ 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 Fcγ 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 FcγRI, FcγRIIa and FcγRIIIa, while those formed by IgG2 bind preferentially to FcγRIIa and FcγRIIIa (Bruhns et al., 2009; Liu, 2015; Tay et al., 2016). In our study, blocking of different FcγRs had differing blocking effects on the occurrence of ADE, which also differed for IgG1 and IgG3 mAbs: FcγRI > FcγRII > FcγRIII, and FcγRIII > FcγRI > FcγRII, respectively (Fig 3C, D). These data are consistent with different binding affinities of antibody subclasses to each type of Fcγ receptor. Blocking of FcγRI binding effectively abrogated ADE caused by the highly interacting subclass IgG1 (mAbs BDBV41, BDBV43, BDBV52) (Fig. 3D) and less by subclass IgG3 antibodies (mAb BDBV223) (Fig. 3C). However, blocking of FcγRIII led to elimination of ADE mediated by IgG3 but not IgG1 antibodies (Fig. 3D). A possible explanation for these data is the low expression of FcγRIII receptors in THP-1 cells and high expression of FcγRI (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 FcγRI and FcγRII; the affinity of interactions of FcγRII with IgGs is much lower (Swisher and Feldman, 2015). Interestingly, in K562 monocytic cells bearing only FcγRII, 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 FcγR.

The LALA mutation of IgG1 mAbs BDBV41 and BDBV43 only slightly decreased ADE (Fig. 3A). The LALA mutation initially was suggested to completely abolish binding to FcγRI (Hezareh et al., 2001), but later was shown to reduce binding to FcγRIIa and FcγRIIIa but not FcγRI (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 FcγRI and the results of blocking FcγRs on induction of ADE by IgG1 (Fig. 3B). Similarly, the KA mutation did not affect the level of ADE (Fig. 3A). This mutation disables antibody-mediated complement activation (Thommesen et al., 2000) but only slightly reduces the binding affinity to FcγRIIa or FcγRIIIa and does not affect binding to FcγRI (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 β1 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 hours after infection with EBOV/BDBV-GP at 1.0 PFU/cell, the percentages of infected U937 cells varied from 0.5 to 1.3% (Fig. 1C), but for THP-1 cells varied from 3 to 8% (Fig. 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 (Fig. 1C).

In humans, FcγRs have been detected on T- and B-lymphocytes, 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 (Fig. 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 (Durbin 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 (Fig. 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 FcγRIIb, ranging from 1 to 48% (Boruchov et al., 2005). It is known that FcγRIIb 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 (Fig. 4) likely is explained by the extreme variability of expression of the inhibitory FcγRIIb in primary human monocytes (Boruchov et al., 2005) and the limitation of phagocytosis by FcγRIIb (Liu et al., 2006). Similarly, the extreme donor-to-donor variability in the levels of FcγRs 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 μM (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 (Fig. 3E, S4C), and Fc-mediated effects prevent effective protection mediated by the same mAb *in vivo* (Fig. 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; Qiu et al., 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 Fcγ receptors may be desirable. However, the Fc-FcγR 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-FcγR 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 * METHOS

## 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 hour-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% CO2 for three days, and plaques were counted using a fluorescent microscope. The promonocytic 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% CO2. 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). $CD14^+$ 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., Cell 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 $F(ab')_2$ 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% $CO_2$. At 48 hours (or 0 or 24 hours where indicated) after inoculation, cells were harvested by low-speed centrifugation (200 × *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% $CO_2$. 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% CO2. At 48 hours after infection, cell suspensions were centrifuged at 200 × *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% $CO_2$. At 24 hours after infection, cells were harvested by low-speed centrifugation (200 × *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% $CO_2$. 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 the approved standard operation procedure. Then, fixed cells were centrifuged at 450 × *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, Inc.). The percentages of $eGFP^+$ 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, staring 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 X100 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 X100 (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 Fc$\gamma$RIII (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 X100. 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 μg/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.

### 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 (Fig. 4), statistical significance was determined using the Holm-Sidak method, with $\alpha = 5\%$. For analysis of survival data in in vivo experiments (Fig. 5), log-rank (Mantel-Cox) test was used.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGEMENS

We acknowledge the excellent technical support of Ms. Xiaoli Shen. This project received support from the U.S. NIH grant U19 AI109711 (to JEC and AB) and Defense Threat Reduction Agency grant HDTRA1-13-1-0034 (to JEC and AB). We are grateful to Dr. Larry Zeitlin for providing plant-derived antibody BDBV43.

## REFERENCES


Acosta EG, and Bartenschlager R (2016). Paradoxical role of antibodies in dengue virus infections: considerations for prophylactic vaccine development. Expert Rev Vaccines 15, 467–482. [PubMed: 26577689]

Ana-Sosa-Batiz F, Johnston AP, Liu H, Center RJ, Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Kim JH, Michael NL, et al. (2014). HIV-specific antibody- dependent phagocytosis matures during HIV infection. Immunol Cell Biol 92, 679–687. [PubMed: 24913323]

Ana-Sosa-Batiz F, Vanderven H, Jegaskanda S, Johnston A, Rockman S, Laurie K, Barr I, Reading P, Lichtfuss M, and Kent SJ (2016). Influenza-Specific Antibody- Dependent Phagocytosis. PLoS One 11, e0154461. [PubMed: 27124730]

Aye KS, Charngkaew K, Win N, Wai KZ, Moe K, Punyadee N, Thiemmeca S, Suttitheptumrong A, Sukpanichnant S, Prida M, et al. (2014). Pathologic highlights of dengue hemorrhagic fever in 13 autopsy cases from Myanmar. Hum Pathol 45, 1221–1233. [PubMed: 24767772]

Baize S, Leroy EM, Georges-Courbot MC, Capron M, Lansoud-Soukate J, Debre P, Fisher-Hoch SP, McCormick JB, and Georges AJ (1999). Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus-infected patients. Nat Med 5, 423–426. [PubMed: 10202932]

Bharat TA, Noda T, Riches JD, Kraehling V, Kolesnikova L, Becker S, Kawaoka Y, and Briggs JA (2012). Structural dissection of Ebola virus and its assembly determinants using cryo-electron tomography. Proceedings of the National Academy of Sciences of the United States of America 109, 4275–4280. [PubMed: 22371572]

Boonnak K, Dambach KM, Donofrio GC, Tassaneetrithep B, and Marovich MA (2011). Cell type specificity and host genetic polymorphisms influence antibody-dependent enhancement of dengue virus infection. J Virol 85, 1671–1683. [PubMed: 21123382]

Bornholdt ZA, Turner HL, Murin CD, Li W, Sok D, Souders CA, Piper AE, Goff A, Shamblin JD, Wollen SE, et al. (2016). Isolation of potent neutralizing antibodies from a survivor of the 2014 Ebola virus outbreak. Science 351, 1078–1083. [PubMed: 26912366]

Borrok MJ, Jung ST, Kang TH, Monzingo AF, and Georgiou G (2012). Revisiting the role of glycosylation in the structure of human IgG Fc. ACS Chem Biol 7, 1596–1602. [PubMed: 22747430]

Boruchov AM, Heller G, Veri MC, Bonvini E, Ravetch JV, and Young JW (2005). Activating and inhibitory IgG Fc receptors on human DCs mediate opposing functions. J Clin Invest 115, 2914–2923. [PubMed: 16167082]

Bournazos S, and Ravetch JV (2017). Fcgamma Receptor Function and the Design of Vaccination Strategies. Immunity 47, 224–233. [PubMed: 28813656]

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. [PubMed: 9728532]

Bruhns P, Iannascoli B, England P, Mancardi DA, Fernandez N, Jorieux S, and Daeron M (2009). Specificity and affinity of human Fcgamma receptors and their polymorphic variants for human IgG subclasses. Blood 113, 3716–3725. [PubMed: 19018092]

Burk R, Bollinger L, Johnson JC, Wada J, Radoshitzky SR, Palacios G, Bavari S, Jahrling PB, and Kuhn JH (2016). Neglected filoviruses. FEMS Microbiol Rev 40, 494–519. [PubMed: 27268907]

Chan KR, Ong EZ, Mok DZ, and Ooi EE (2015). Fc receptors and their influence on efficacy of therapeutic antibodies for treatment of viral diseases. Expert Rev Anti Infect Ther 13, 1351–1360. [PubMed: 26466016]

Clapham HE, Quyen TH, Kien DT, Dorigatti I, Simmons CP, and Ferguson NM (2016). Modelling Virus and Antibody Dynamics during Dengue Virus Infection Suggests a Role for Antibody in Virus Clearance. PLoS Comput Biol 12, e1004951. [PubMed: 27213681]

Corti D, Misasi J, Mulangu S, Stanley DA, Kanekiyo M, Wollen S, Ploquin A, Doria-Rose NA, Staupe RP, Bailey M, et al. (2016). Protective monotherapy against lethal Ebola virus infection by a potently neutralizing antibody. Science 351, 1343–1346. [PubMed: 26917592]

Davidson E, Bryan C, Fong RH, Barnes T, Pfaff JM, Mabila M, Rucker JB, and Doranz BJ (2015). Mechanism of Binding to Ebola Virus Glycoprotein by the ZMapp, ZMAb, and MB-003 Cocktail Antibodies. J Virol 89, 10982–10992. [PubMed: 26311869]

Dube D, Schornberg KL, Stantchev TS, Bonaparte MI, Delos SE, Bouton AH, Broder CC, and White JM (2008). Cell adhesion promotes Ebola virus envelope glycoprotein- mediated binding and infection. J Virol 82, 7238–7242. [PubMed: 18448524]

Durbin AP, Vargas MJ, Wanionek K, Hammond SN, 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. [PubMed: 18452966]

Dye JM, Herbert AS, Kuehne AI, Barth JF, Muhammad MA, Zak SE, Ortiz RA, Prugar LI, and Pratt WD (2012). Postexposure antibody prophylaxis protects nonhuman primates from filovirus disease. Proceedings of the National Academy of Sciences of the United States of America 109, 5034–5039. [PubMed: 22411795]

Fibriansah G, Ibarra KD, Ng TS, Smith SA, Tan JL, Lim XN, Ooi JS, Kostyuchenko VA, Wang J, de Silva AM, 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. [PubMed: 26138979]

Fleit HB, and Kobasiuk CD (1991). The human monocyte-like cell line THP-1 expresses Fc gamma RI and Fc gamma RII. J Leukoc Biol 49, 556–565. [PubMed: 1709200]

Flipse J, Wilschut J, and Smit JM (2013). Molecular mechanisms involved in antibody- dependent enhancement of dengue virus infection in humans. Traffic 14, 25–35. [PubMed: 22998156]

Flyak AI, Ilinykh PA, Murin CD, Garron T, Shen X, Fusco ML, Hashiguchi T, Bornholdt ZA, Slaughter JC, Sapparapu G, et al. (2015). Mechanism of human antibody- mediated neutralization of Marburg virus. Cell 160, 893–903. [PubMed: 25723164]

Flyak AI, Kuzmina N, Murin CD, Bryan C, Davidson E, Gilchuk P, Gulka CP, Ilinykh PA, 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.

Flyak AI, Shen X, Murin CD, Turner HL, David JA, Fusco ML, Lampley R, Kose N, Ilinykh PA, Kuzmina N, et al. (2016). Cross-Reactive and Potent Neutralizing Antibody Responses in Human Survivors of Natural Ebolavirus Infection. Cell 164, 392–405. [PubMed: 26806128]

Furuyama W, Marzi A, Carmody AB, Maruyama J, Kuroda M, Miyamoto H, Nanbo A, Manzoor R, Yoshida R, Igarashi M, et al. (2016). Fcgamma-receptor IIa-mediated Src Signaling Pathway is Essential for the Antibody-Dependent Enhancement of Ebola Virus Infection. PLoS Pathog 12, e1006139. [PubMed: 28036370]

Geisbert TW, Hensley LE, Larsen T, Young HA, Reed DS, Geisbert JB, Scott DP, Kagan E, Jahrling PB, and Davis KJ (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. [PubMed: 14633608]

Gilchuk P, Kuzmina NA, Ilinykh PA, Huang K, Gunn BM, Bryan A, Davidson E, Doranz BJ, Turner HL, Fusco ML, et al. (2018). Multifunctional pan-ebolavirus antibodies recognize a site of broad vulnerability on the ebolavirus glycoprotein. Immunity IMMUNITY-D-18–00300R1 in press.

Gillis C, Gouel-Cheron A, Jonsson F, and Bruhns P (2014). Contribution of Human FcgammaRs to Disease with Evidence from Human Polymorphisms and Transgenic Animal Studies. Front Immunol 5, 254. [PubMed: 24910634]

Gollins SW, and Porterfield JS (1985). Flavivirus infection enhancement in macrophages: an electron microscopic study of viral cellular entry. J Gen Virol 66 (Pt 9), 1969–1982. [PubMed: 4031825]

Halstead SB (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, 493–516.

Halstead SB, and O'Rourke EJ (1977). Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J Exp Med 146, 201–217. [PubMed: 406347]

Halstead SB, O'Rourke EJ, and Allison AC (1977). Dengue viruses and mononuclear phagocytes. II. Identity of blood and tissue leukocytes supporting in vitro infection. J Exp Med 146, 218–229. [PubMed: 195000]

Henao-Restrepo AM, Longini IM, Egger M, Dean NE, Edmunds WJ, Camacho A, Carroll MW, 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. [PubMed: 26248676]

Hessell AJ, Hangartner L, Hunter M, Havenith CE, Beurskens FJ, Bakker JM, Lanigan CM, Landucci G, Forthal DN, Parren PW, et al. (2007). Fc receptor but not complement binding is important in antibody protection against HIV. Nature 449, 101–104. [PubMed: 17805298]

Hezareh M, Hessell AJ, Jensen RC, van de Winkel JG, and Parren PW (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. [PubMed: 11711607]

Higel F, Seidl A, Sorgel 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. [PubMed: 26775146]

Hogarth PM, and Pietersz GA (2012). Fc receptor-targeted therapies for the treatment of inflammation, cancer and beyond. Nat Rev Drug Discov 11, 311–331. [PubMed: 22460124]

Ilinykh PA, Shen X, Flyak AI, Kuzmina N, Ksiazek TG, Crowe JE Jr., and Bukreyev A (2016). Chimeric Filoviruses for Identification and Characterization of Monoclonal Antibodies. J Virol 90, 3890–3901. [PubMed: 26819310]

Jahrling PB, Geisbert JB, Swearengen JR, Larsen T, and Geisbert TW (2007). Ebola hemorrhagic fever: evaluation of passive immunotherapy in nonhuman primates. J Infect Dis 196 Suppl 2, S400–403. [PubMed: 17940976]

Kou Z, Lim JY, Beltramello M, Quinn M, Chen H, Liu S, Martinez-Sobrido L, Diamond MS, Schlesinger JJ, 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. [PubMed: 21131015]

Ksiazek TG, Rollin PE, Williams AJ, Bressler DS, Martin ML, Swanepoel R, Burt FJ, Leman PA, Khan AS, Rowe AK, et al. (1999). Clinical virology of Ebola hemorrhagic fever (EHF): virus, virus antigen, and IgG and IgM antibody findings among EHF patients in Kikwit, Democratic Republic of the Congo, 1995. J Infect Dis 179 Suppl 1, S177–187. [PubMed: 9988182]

Kudoyarova-Zubavichene NM, Sergeyev NN, Chepurnov AA, and Netesov SV (1999). Preparation and use of hyperimmune serum for prophylaxis and therapy of Ebola virus infections. J Infect Dis 179 Suppl 1, S218–223. [PubMed: 9988187]

Kuhn JH (2008). Filoviruses, 1 edn (Wien New York: Springer).

Lee JE, Fusco ML, Hessell AJ, Oswald WB, Burton DR, and Saphire EO (2008). Structure of the Ebola virus glycoprotein bound to an antibody from a human survivor. Nature 454, 177–182. [PubMed: 18615077]

Littaua R, Kurane I, and Ennis FA (1990). Human IgG Fc receptor II mediates antibody- dependent enhancement of dengue virus infection. J Immunol 144, 3183–3186. [PubMed: 2139079]

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. [PubMed: 25872915]

Liu Y, Gao X, Masuda E, Redecha PB, Blank MC, and Pricop L (2006). Regulated expression of FcgammaR in human dendritic cells controls cross-presentation of antigen- antibody complexes. J Immunol 177, 8440–8447. [PubMed: 17142741]

Lubaki NM, Ilinykh P, Pietzsch C, Tigabu B, Freiberg AN, Koup RA, 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. Journal of virology 87, 7471–7485. [PubMed: 23616668]

Lubaki NM, Younan P, Santos RI, Meyer M, Iampietro M, Koup RA, and Bukreyev A (2016). The Ebola Interferon Inhibiting Domains Attenuate and Dysregulate Cell-Mediated Immune Responses. PLoS Pathog 12, e1006031. [PubMed: 27930745]

Martinez O, Johnson JC, Honko A, Yen B, Shabman RS, Hensley LE, Olinger GG, and Basler CF (2013). Ebola virus exploits a monocyte differentiation program to promote its entry. J Virol 87, 3801–3814. [PubMed: 23345511]

Martins KA, Jahrling PB, Bavari S, and Kuhn JH (2016). Ebola virus disease candidate vaccines under evaluation in clinical trials. Expert Rev Vaccines 15, 1101–1112. [PubMed: 27160784]

McElroy AK, Akondy RS, Davis CW, Ellebedy AH, Mehta AK, Kraft CS, Lyon GM, Ribner BS, Varkey J, Sidney J, et al. (2015). Human Ebola virus infection results in substantial immune activation. Proc Natl Acad Sci U S A 112, 4719–4724. [PubMed: 25775592]

Messaoudi I, Amarasinghe GK, and Basler CF (2015). Filovirus pathogenesis and immune evasion: insights from Ebola virus and Marburg virus. Nat Rev Microbiol 13, 663–676. [PubMed: 26439085]

Mire CE, Geisbert JB, Borisevich V, Fenton KA, Agans KN, Flyak AI, Deer DJ, 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, pii: eaai8711.

Morens DM, Venkateshan CN, and Halstead SB (1987). Dengue 4 virus monoclonal antibodies identify epitopes that mediate immune infection enhancement of dengue 2 viruses. J Gen Virol 68 (Pt 1), 91–98. [PubMed: 2433388]

Murin CD, Fusco ML, Bornholdt ZA, Qiu X, Olinger GG, Zeitlin L, Kobinger GP, Ward AB, and Saphire EO (2014). Structures of protective antibodies reveal sites of vulnerability on Ebola virus. Proc Natl Acad Sci U S A 111, 17182–17187. [PubMed: 25404321]

Nakayama E, Tomabechi D, Matsuno K, Kishida N, Yoshida R, Feldmann H, and Takada A (2011). Antibody-dependent enhancement of marburg virus infection. The Journal of infectious diseases 204 Suppl 3, S978–985. [PubMed: 21987779]

Nimmerjahn F, and Ravetch JV (2008). Fcgamma receptors as regulators of immune responses. Nat Rev Immunol 8, 34–47. [PubMed: 18064051]

Olinger GG Jr., Pettitt J, Kim D, Working C, Bohorov O, Bratcher B, Hiatt E, Hume SD, Johnson AK, 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 U S A 109, 18030–18035. [PubMed: 23071322]

Oswald WB, Geisbert TW, Davis KJ, Geisbert JB, Sullivan NJ, Jahrling PB, Parren PW, and Burton DR (2007). Neutralizing Antibody Fails to Impact the Course of Ebola Virus Infection in Monkeys. PLoS Pathog 3, e9. [PubMed: 17238286]

Park JG, Isaacs RE, Chien P, and Schreiber AD (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. [PubMed: 8408649]

Pyankov OV, Setoh YX, Bodnev SA, Edmonds JH, Pyankova OG, Pyankov SA, 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. [PubMed: 28155869]

Qiu X, Alimonti JB, Melito PL, Fernando L, Ströher U, and Jones SM (2011). Characterization of Zaire ebolavirus glycoprotein-specific monoclonal antibodies. Clinical immunology.

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, 329ra333.

Qiu X, Audet J, Wong G, Pillet S, Bello A, Cabral T, Strong JE, Plummer F, Corbett CR, Alimonti JB, et al. (2012). Successful treatment of Ebola virus-infected cynomolgus macaques with monoclonal antibodies. Science translational medicine 4, 138ra181.

Qiu X, Wong G, Audet J, Bello A, Fernando L, Alimonti JB, 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. [PubMed: 25171469]

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. [PubMed: 26522769]

Reusch D, and Tejada ML (2015). Fc glycans of therapeutic antibodies as critical quality attributes. Glycobiology 25, 1325–1334. [PubMed: 26263923]

Rodrigo WW, Jin X, Blackley SD, Rose RC, and Schlesinger JJ (2006). Differential enhancement of dengue virus immune complex infectivity mediated by signaling-competent and signaling-

incompetent human Fcgamma RIA (CD64) or FcgammaRIIA (CD32). J Virol 80, 10128–10138. [PubMed: 17005690]

Sanchez A, Trappier SG, Mahy BW, Peters CJ, and Nichol ST (1996). The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. Proc Natl Acad Sci U S A 93, 3602–3607. [PubMed: 8622982]

Ströher U, West E, Bugany H, Klenk HD, Schnittler HJ, and Feldmann H (2001). Infection and activation of monocytes by Marburg and Ebola viruses. J Virol 75, 11025–11033. [PubMed: 11602743]

Swisher JF, and Feldman GM (2015). The many faces of FcgammaRI: implications for therapeutic antibody function. Immunol Rev 268, 160–174. [PubMed: 26497519]

Takada A, Ebihara H, Feldmann H, Geisbert TW, and Kawaoka Y (2007). Epitopes required for antibody-dependent enhancement of Ebola virus infection. J Infect Dis 196 Suppl 2, S347–356. [PubMed: 17940970]

Takada A, Feldmann H, Ksiazek TG, and Kawaoka Y (2003). Antibody-dependent enhancement of Ebola virus infection. J Virol 77, 7539–7544. [PubMed: 12805454]

Takada A, Watanabe S, Okazaki K, Kida H, and Kawaoka Y (2001). Infectivity-enhancing antibodies to Ebola virus glycoprotein. J Virol 75, 2324–2330. [PubMed: 11160735]

Tay MZ, Liu P, Williams LD, McRaven MD, Sawant S, Gurley TC, Xu TT, Dennison SM, Liao HX, Chenine AL, et al. (2016). Antibody-Mediated Internalization of Infectious HIV-1 Virions Differs among Antibody Isotypes and Subclasses. PLoS Pathog 12, e1005817. [PubMed: 27579713]

Thommesen JE, Michaelsen TE, Loset GA, Sandlie I, and Brekke OH (2000). Lysine 322 in the human IgG3 C(H)2 domain is crucial for antibody dependent complement activation. Mol Immunol 37, 995–1004. [PubMed: 11395138]

Volchkov VE, Becker S, Volchkova VA, Ternovoj VA, Kotov AN, Netesov SV, and Klenk HD (1995). GP mRNA of Ebola virus is edited by the Ebola virus polymerase and by T7 and vaccinia virus polymerases. Virology 214, 421–430. [PubMed: 8553543]

Williams KL, Sukupolvi-Petty S, Beltramello M, Johnson S, Sallusto F, Lanzavecchia A, Diamond MS, and Harris E (2013). Therapeutic efficacy of antibodies lacking Fcgamma receptor binding against lethal dengue virus infection is due to neutralizing potency and blocking of enhancing antibodies [corrected]. PLoS Pathog 9, e1003157. [PubMed: 23459315]

Wilson JA, Hevey M, Bakken R, Guest S, Bray M, Schmaljohn AL, and Hart MK (2000). Epitopes involved in antibody-mediated protection from Ebola virus. Science 287, 1664–1666. [PubMed: 10698744]

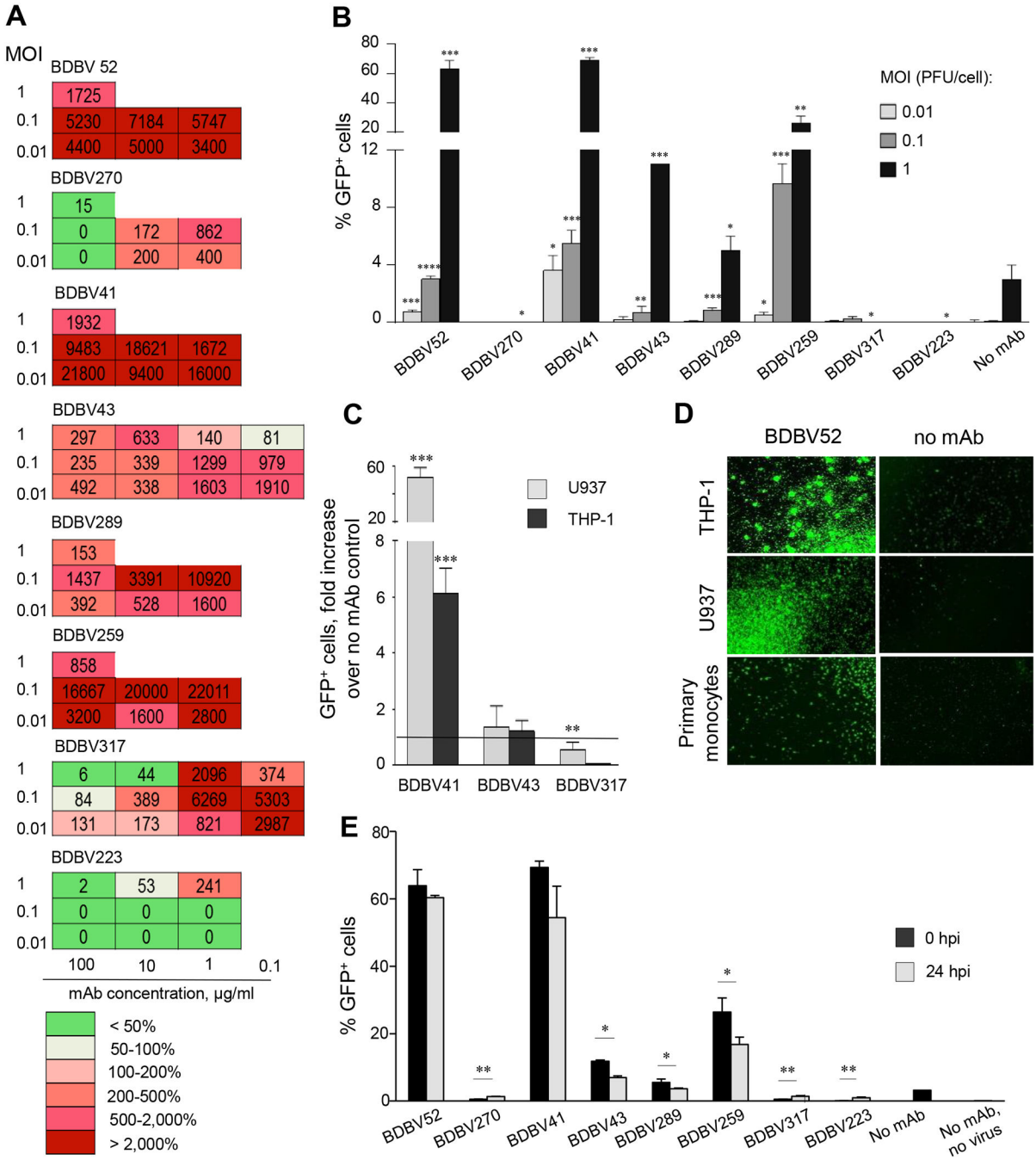


**Figure 1.**

ADE of EBOV/BDBV-GP infection in primary monocytes or monocytic cell lines. A. Heat maps showing percentages of eGFP$^+$ THP-1 cells at differing concentrations of virus or mAbs, normalized to cells with no mAbs added (100%). B. Percentages of eGFP$^+$ THP-1 cells at differing viral doses in the presence of mAbs at 100 µg/mL. C. Fold increase over no mAb control of eGFP$^+$ THP-1 and U937 cells infected at MOI of 1.0 PFU/cell and treated with 100 µg/mL of the indicated mAb. D. Aggregation of the indicated cells inoculated at an MOI of 1.0 PFU/cell and treated with 100 µg/mL of BDBV52 mAb. E. Percentages of eGFP

[+] THP-1 cells infected at an MOI of 1.0 PFU/cell and treated with the indicated mAb at 100 μg/ml immediately after infection (0 hours) or after 24 hours. B, C, E, mean values ±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).

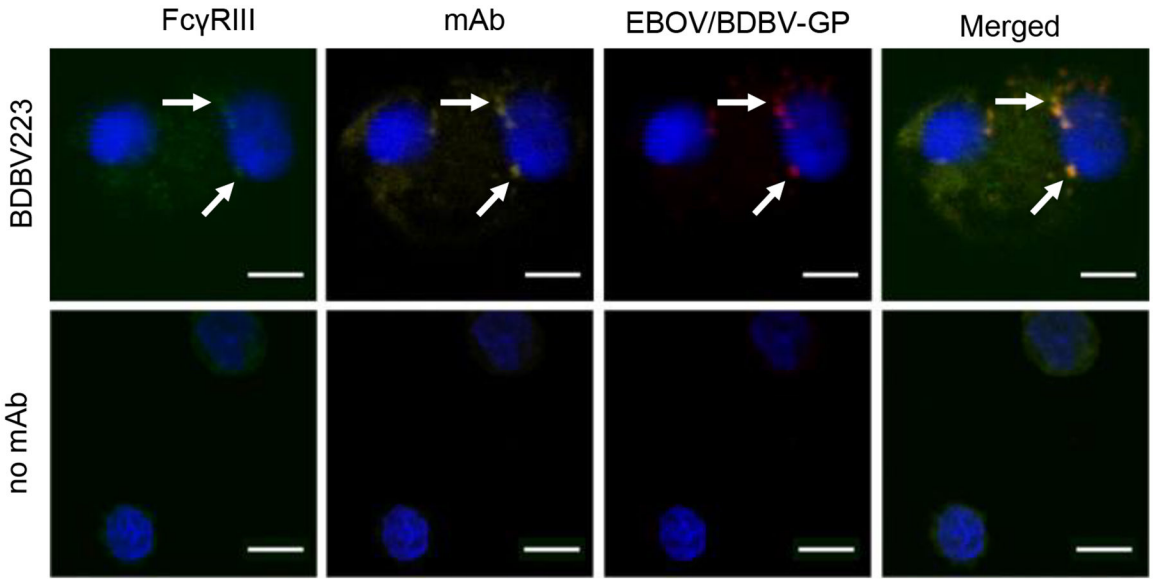


**Fig. 2.**

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 hour after inoculation. FcγRIII are stained with antibodies labeled with AlexaFluor 488 (green), mAbs with Dy594 (orange) or the virus with AlexaFluor 647 (red). Scale bar = 5 μm. Nuclei are stained with DAPI (blue). Arrows point to co-localization of FcγRs, mAb and virus.

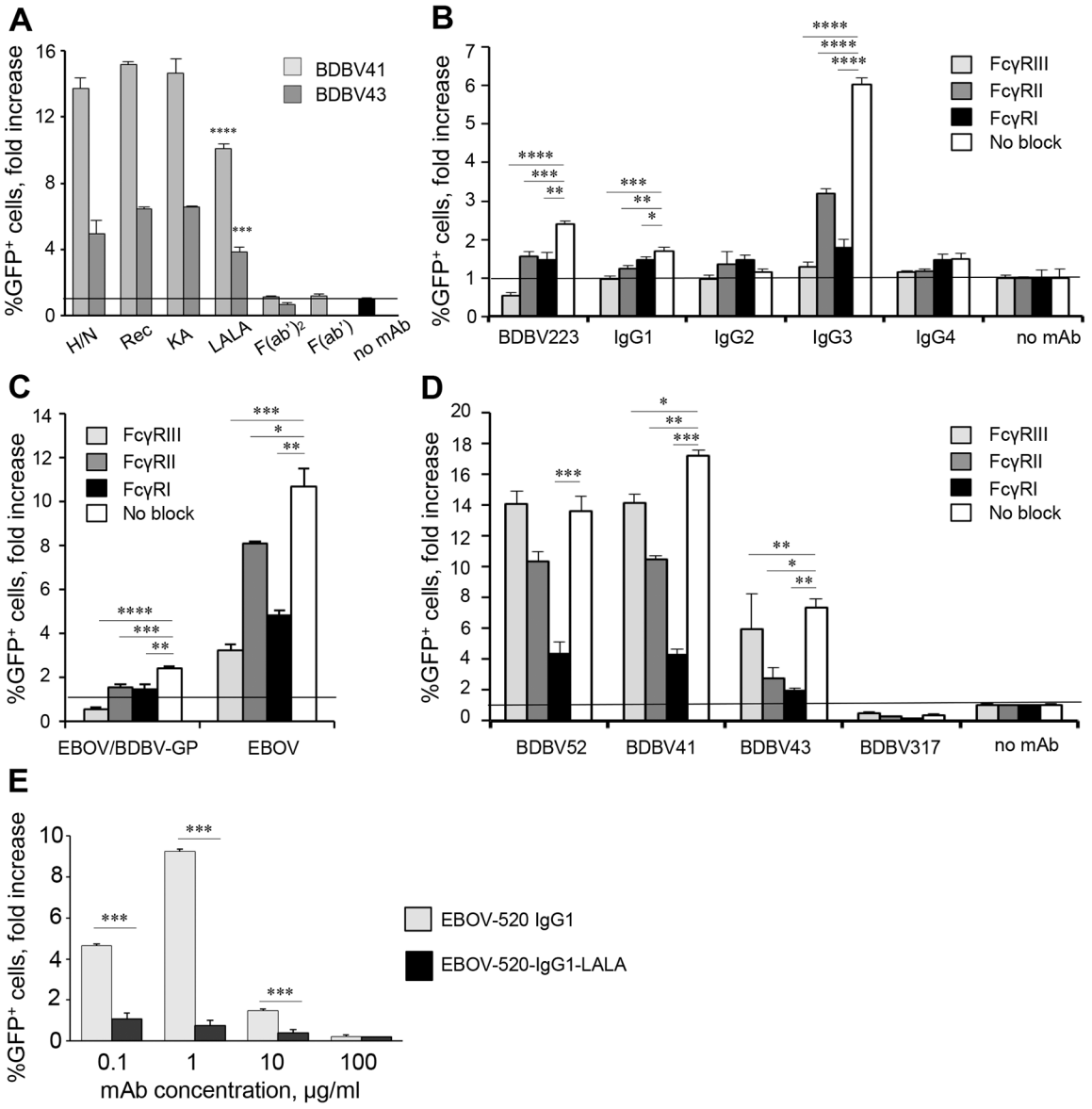


**Fig. 3.**

ADE depends on the presence of Fc receptors, Fc fragments and on the subclass of IgG: fold increases of the percentages of infected (eGFP$^+$) THP-1 cells treated with the indicated filovirus mAb with or without Fcγ**R**-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′)$_2$ 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 Fcγ**R**-blocking antibodies. **C**. Cells inoculated with the indicated viruses and treated with 0.1 μg/ml BDBV223 in presence of the indicated Fcγ**R**-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 (panel A) or for the indicated pairs: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ (Multiple t-test).

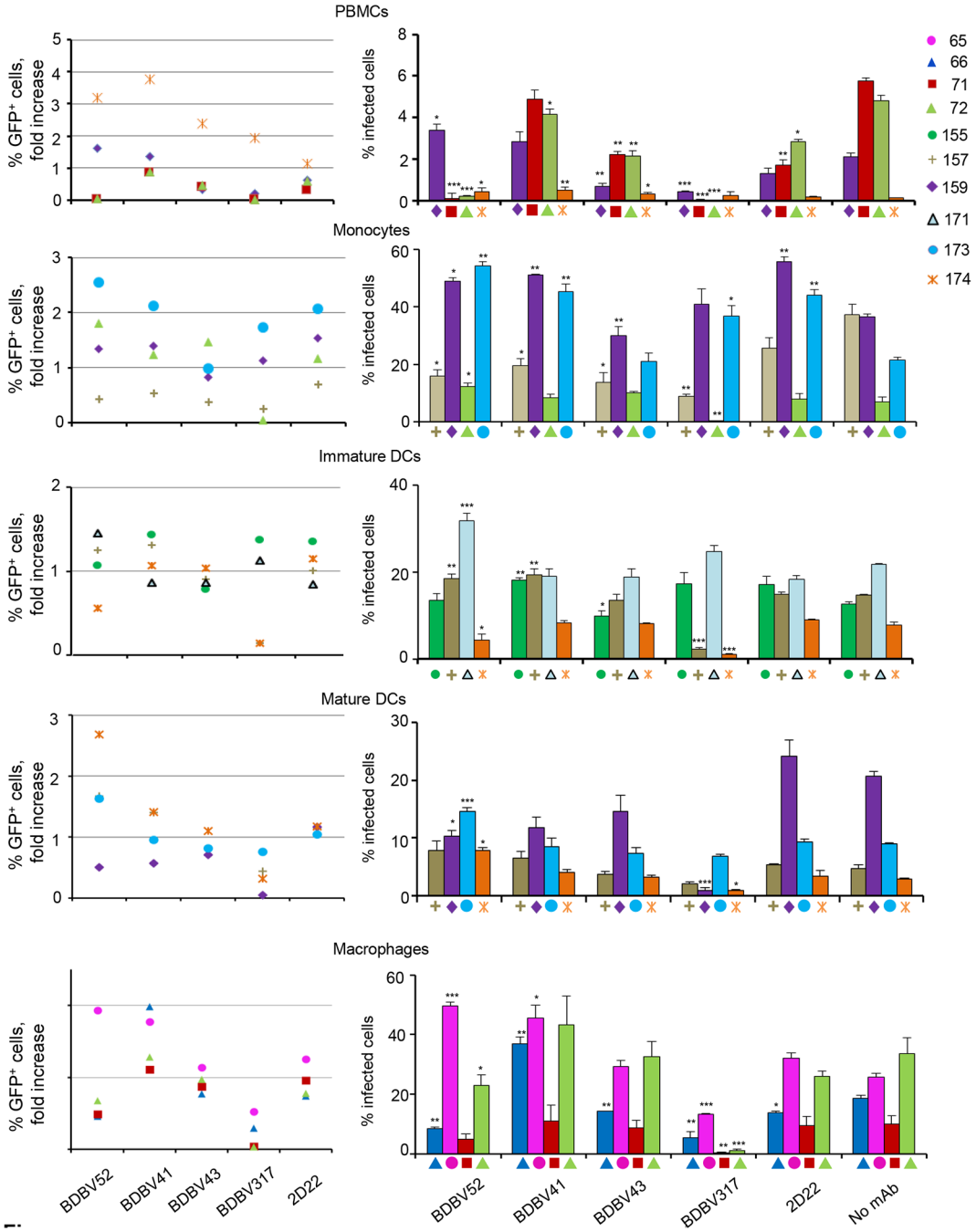


**Fig. 4.**

ADE of EBOV infection in primary human immune cells. Left, fold increase in percentage of infected (eGFP$^+$) cells due to the presence of the filovirus mAb indicated on the x-axis. Right, percentage of infected (eGFP$^+$) cells in the presence or absence of filovirus mAbs indicated on the x-axis, mean values ±SD based on triplicate samples. Cells from individual donors are indicated with various symbols. Differences to no antibody control (Multiple t-test): * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

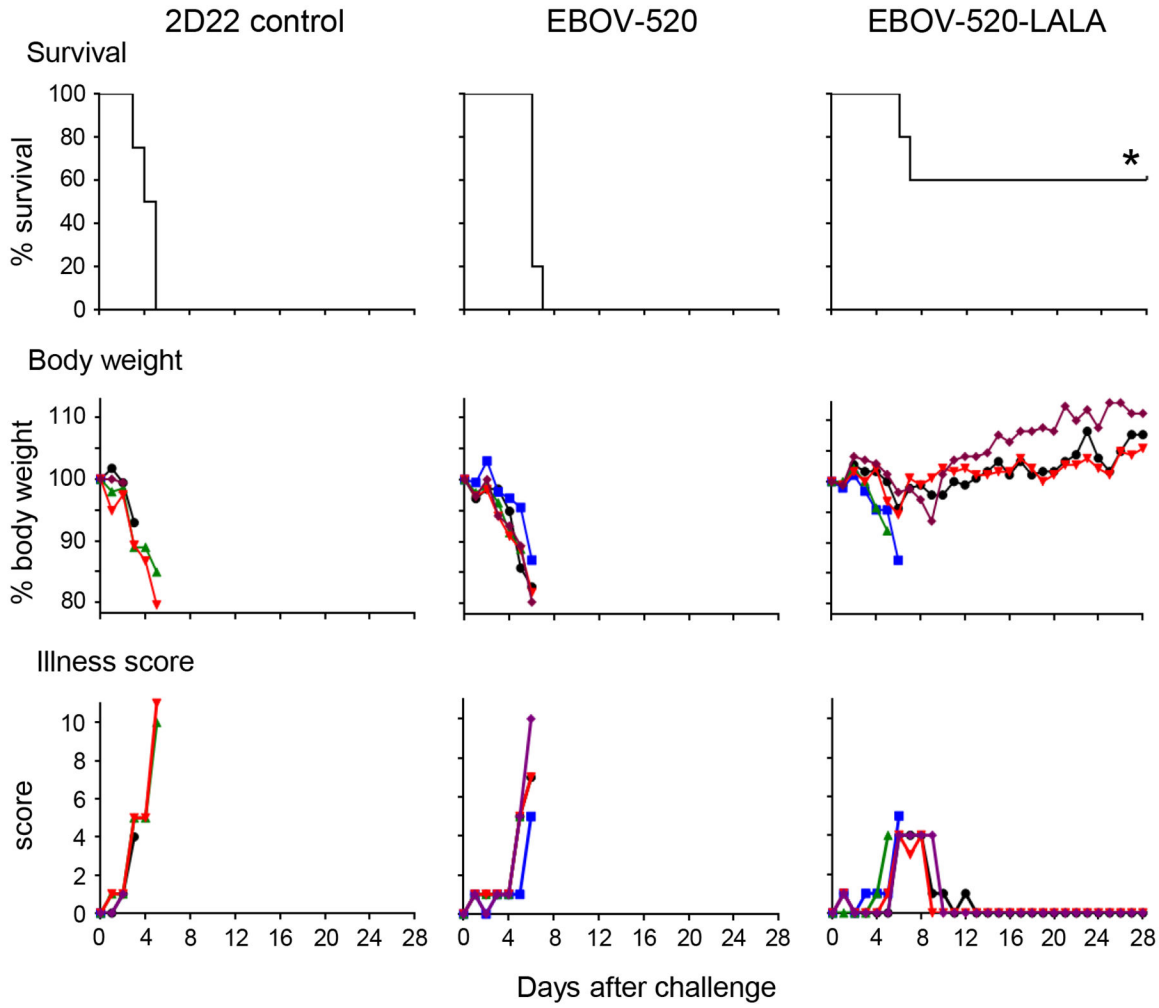


**Fig. 5.**

ADE counteracts antibody-mediated protection *in vivo*. **B**. 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).