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14. ABSTRACT The overall research idea of this proposal is to ascertain the feasibility of providing short-term protection against Ebola virus infection while co-administering Ebola virus vaccines to provide long-term protection against disease. Ebola virus vaccine candidates (a live replicating vesicular stomatitis virus (VSV) expressing Ebola-GP and a replication-deficient adenovirus (AdV) expressing Ebola GP) have been used in humans. For therapeutic purposes, monoclonal antibodies (ZMapp, mAb114) and antiviral small molecules (Favipiravir, Remdesivir) are also being tested in human infections. However, neither antibody therapy nor small molecule inhibitors of Ebola virus replication will protect against infection long-term, and vaccines do not provide immediate protection. To that end, this proposal will test multiple strategies to combine therapeutic drugs with vaccines to generate rapid short-term as well as long-term protection against Ebola virus. In the first year we found that administration of neutralizing antibodies had an effect on the short-term immunogenicity of both VSV and AdV vaccines. There was no significant effect on longer-term antibody responses for the VSV vaccine, and a variable effect for the AdV vaccine. However, there was no inhibition of antibody cocktail levels caused by vaccination. Therefore, the first and second years have demonstrated regimens that may provide both short-and long-term immunity.					
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1. **INTRODUCTION:** Ebola virus causes severe disease and often death in human infections. Recent large outbreaks have demonstrated that the virus is a serious emerging threat to human health. The overall research idea of this proposal is to ascertain the feasibility of providing short-term protection against Ebola virus infection while co-administering Ebola virus vaccines to provide long-term protection against disease. Two Ebola virus vaccine candidates, a live vesicular stomatitis virus expressing Ebola glycoprotein (VSV-GP) and a single-cycle adenovirus vector expressing Ebola glycoprotein (AdV-GP) have been extensively tested in humans, and the VSV-GP has been used as a ring vaccine in Ebola virus outbreaks. The use of monoclonal antibodies has been very promising in nonhuman primate studies and have been used experimentally in human infections, as have the small molecules Favipiravir (T-705) and Remdesivir (GS-5734). However, neither antibody therapy nor small molecule inhibitors of Ebola virus replication will protect against infection long-term, and vaccines do not provide immediate protection. What is needed for military personnel and health care workers responding to filovirus outbreaks is a regimen to protect in the short-term against infection, and in the long-term as well. To that end, this proposal will test multiple strategies to combine therapeutic drugs with vaccines to generate rapid short-term as well as long-term protection against Ebola virus. Specific Aim 1 is to test the effects of time of administration of Ebola virus neutralizing antibody cocktails on immunogenicity of advanced Ebola virus vaccines, and to test how these regimens affect the neutralizing antibody cocktail levels. Specific Aim 2 is to determine whether functional non-neutralizing anti-GP antibodies, and selective anti-sGP antibodies, affect vaccine immunogenicity. Specific Aim 3 will compare the effects of Remdesivir and Favipiravir administration on vaccination. This report focuses on Year 2 of this grant, which comprises Specific Aim 2.
2. **KEYWORDS:** Ebola, virus, vaccination, vaccine, filovirus, antibody, monoclonal, therapeutics, drugs, Remdesivir, Favipiravir, ZMAPP, mAb114, glycoprotein, MIL77, GP.
3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

- Major Task 1: Determine whether functional non-neutralizing anti-GP1,2 antibodies, and selective anti-sGP antibodies, affect vaccine immunogenicity.
 - Subtask 1: Determine effects of protective non-neutralizing antibody administration on vaccine immunogenicity (Months 13-18)
 - 75% accomplished
 - Subtask 2: Determine the effects of anti-sGP antibody administration on vaccine immunogenicity (Months 15-21)
 - Pending identification of sufficient antibody quantity
 - Subtask 3: Assessment of protection against live virus challenge (Months 21-24)
 - Delayed due to COVID-19
 - Milestone 1: Establishment of optimal regimen for administering Ebola vaccines and non-neutralizing antibodies
 - 50% accomplished

- **What was accomplished under these goals?**

a) Major activities

Acquisition of Ebola virus monoclonal antibodies. The goal of this aim was to test how Ebola virus functional but non-neutralizing antibodies affected immunogenicity of two Ebola virus vaccines. We used two well characterized non-neutralizing antibodies, 6D8 and 13c6, both of which were a gift from Dr. John Dye at the United States Army Medical Research Institute of Infectious Diseases. These antibodies were used in the below experiments.

Acquisition of Ebola virus vaccines. We acquired the VSV-GP vaccine through the generosity of Dr. Heinz Feldmann and used this for the mouse experiments. However, we were not able to acquire the ChAd3-GP vaccine, as it is being used in clinical trials and we were not given permission to acquire this vaccine. We therefore contracted the generation of a replication-defective AdV5-GP vaccine, since no ChAd3 backbone was commercially available. We screened several AdV serotype 5-GP viruses by western blot and picked a clone that expressed GP at a high level. We received virus at 5×10^{12} particles/mL, sufficient for the in vivo experiments.

Assessment of the effects of vaccination on Ebola virus vaccine immunogenicity and the effects of vaccines on monoclonal antibody persistence. The results of these experiments are described in subsection c below.

b) specific objectives

Major Task 1: Determine whether functional non-neutralizing anti-GP1,2 antibodies, and selective anti-sGP antibodies, affect vaccine immunogenicity.

Subtask 1: Determine effects of protective non-neutralizing antibody administration on vaccine immunogenicity (Months 13-18)

Subtask 2: Determine the effects of anti-sGP antibody administration on vaccine immunogenicity (Months 15-21)

Subtask 3: Assessment of protection against live virus challenge (Months 21-24)

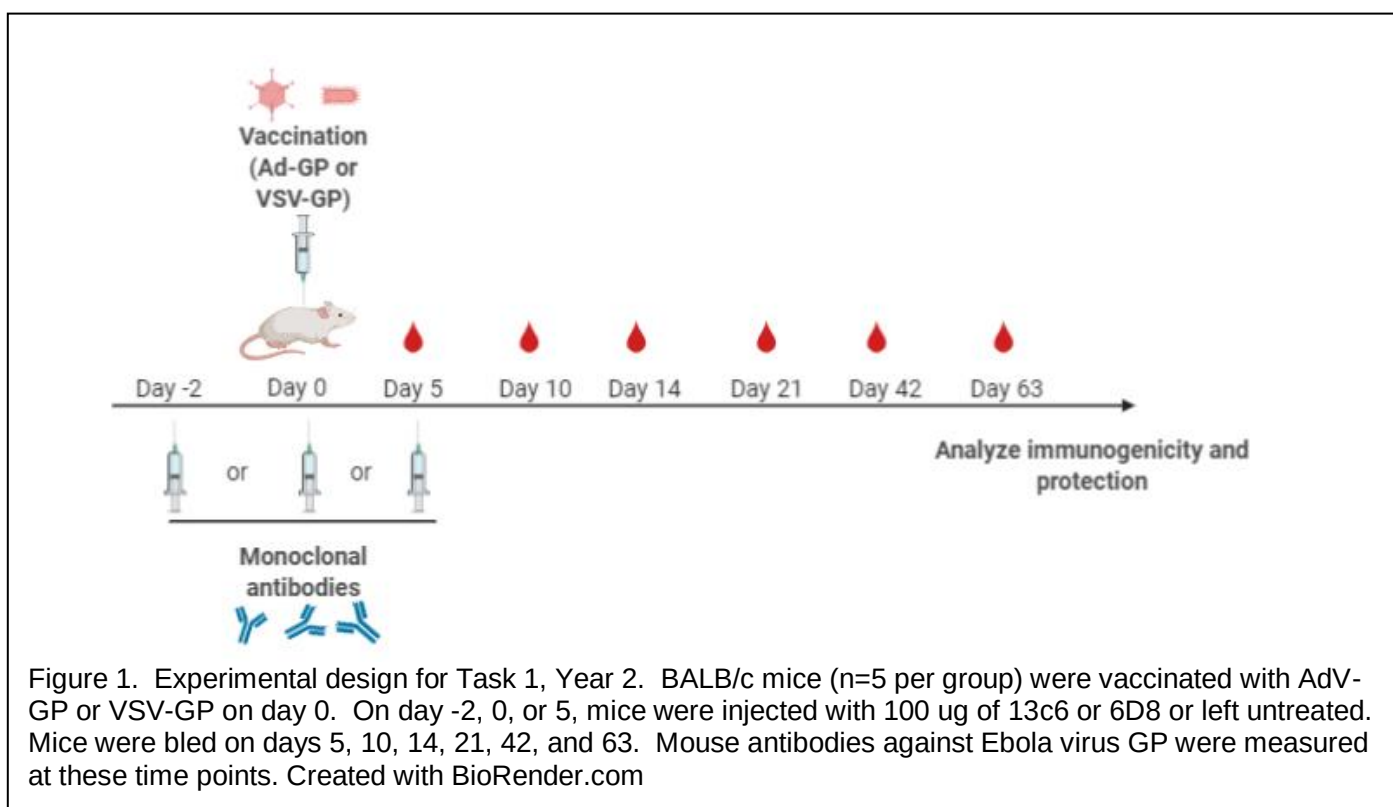
c) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)

Major Task 1: Determine whether functional non-neutralizing anti-GP1,2 antibodies, and selective anti-sGP antibodies, affect vaccine immunogenicity.

Subtask 1: Determine effects of protective non-neutralizing antibody administration on vaccine immunogenicity

Methods. BALB/c mice (n=5 per group) were vaccinated intramuscularly with either 2×10^4 pfu of VSV-GP, 1×10^9 viral particles of Ad-GP, or mock vaccinated (as a negative control) on day 0. The functional non-neutralizing murine antibodies 6D8 and 13c6 were injected intravenously with 100 ug of each antibody treatment on days -2, 0, and +5 (as a control, for each vaccine one group was vaccinated but not injected with antibody). Mice were bled via the submandibular route on days 5, 10, 14, 21, 42, and 63 to assay for vaccine-induced antibody responses. ELISAs were conducted on a 1:40 dilution of sera for anti-Ebola GP IgG and IgM mouse antibody for days 5, 10, 14, 21, 42 and 63 (Figure 1). The 13c6 and 6D8 are both IgG isotype IgG2a, and so levels of IgG recorded both administered murine antibody and induced IgG. IgM antibodies were assayed by ELISA to demonstrate the response solely from the vaccinated mice. ELISAs were performed using protocols established by the Bradfute laboratory (as detailed in the Year 1 report).

Statistical analysis. Antibody levels were compared to vaccine-only groups with vaccine+antibody groups using a one-way ANOVA with Dunnett's multiple comparisons test. A p value of <0.05 was considered to be significant.



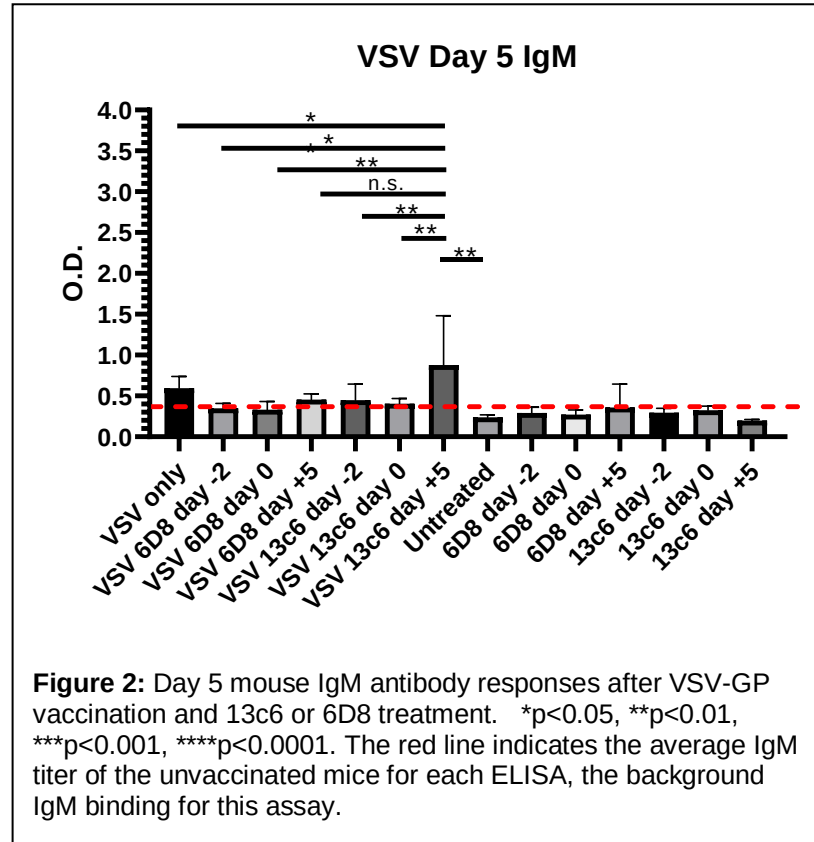
Results.

VSV-GP vaccination

IgM titers after VSV-GP vaccination.

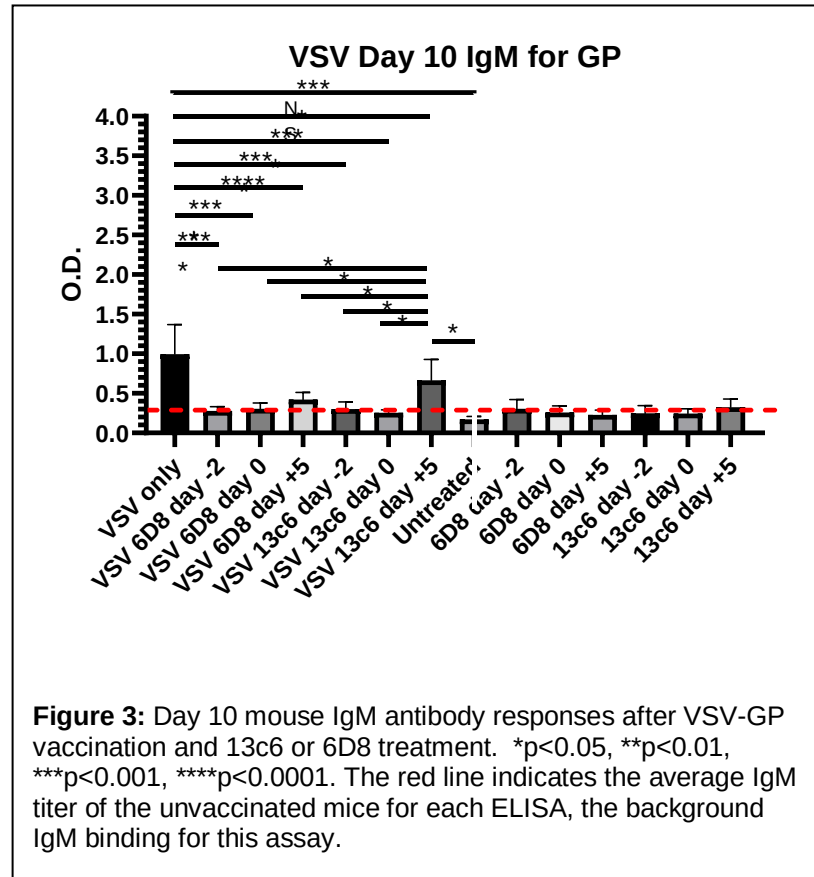
Mice receiving VSV-GP on day 0 and treated with 13c6 or 6D8 on days -2, 0, or 5 were bled on days 5, 10, 14 and 21. As IgM has been shown to be a major contributor to VSV-GP-generated functional antibodies (Khurana et al, 2016). ELISAs were performed with the sera to assay IgM levels. The anti-mouse IgM secondary antibody was cross-absorbed to prevent binding to IgG, which should prevent the administered antibodies, which are IgG isotype, from being non-specifically detected by the anti-IgM secondary. To further control for this, the unvaccinated controls

were also treated with antibodies at the same times as the vaccinated groups (the unvaccinated groups are listed in the figures as untreated, 6D8 -2, 6D8 0, 6D8 5, 13c6 -2, 13c6 0 and 13c6 5) to show there is no IgM background or non-specific binding resulting from the administration of the antibodies.



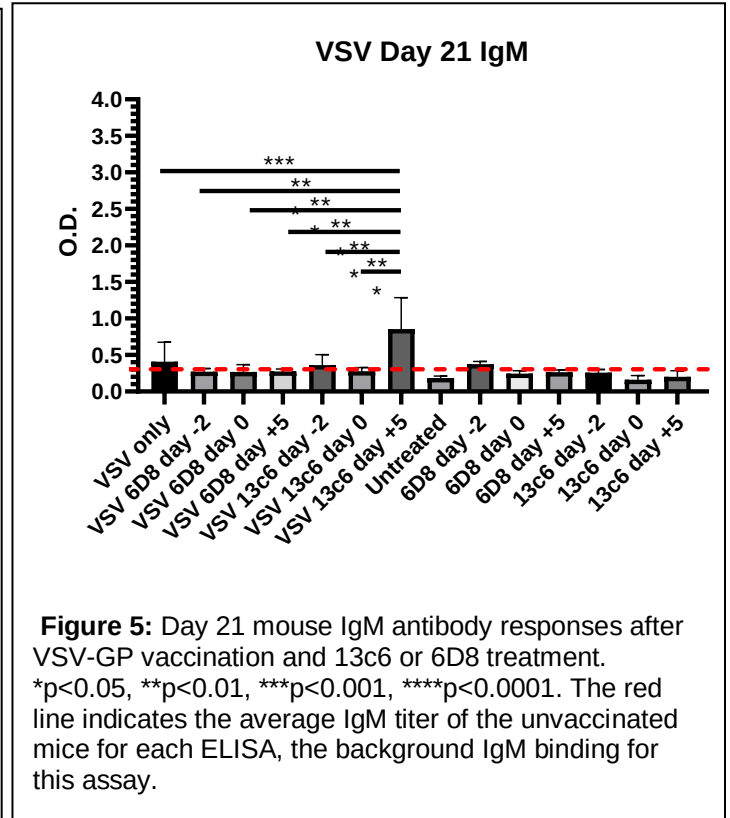
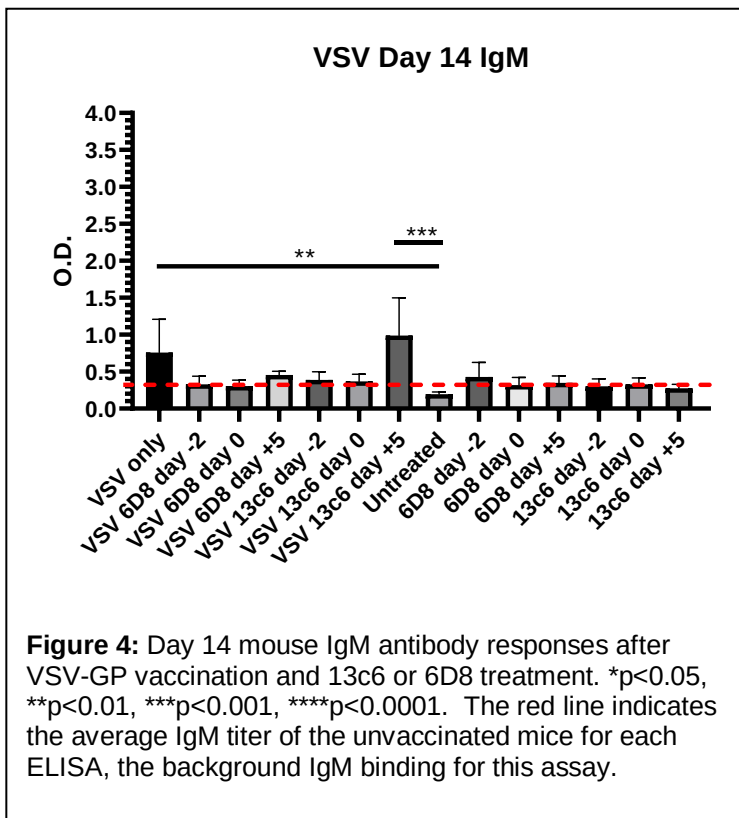
On day 5, there was a mouse IgM anti-Ebola GP response detected by ELISA in the VSV-GP vaccinated group, but it was not significantly different compared to the unvaccinated. However, VSV-GP + 13c6 administered on day 5 group (VSV 13c6 d5) induced significant vaccine-elicited IgM compared to untreated/unvaccinated mice and compared to the VSV vaccine only group.

At day 10, significant IgM titers were present in the VSV-GP vaccine only group, and administration of 13c6 and 6D8 on days -2 and 0 significantly diminished the mouse IgM anti-GP responses. However, administration of 13c6 on day 5 did not significantly diminish the mouse anti-GP IgM response. At day 10, there was a significant difference between the VSV-GP vaccine only and the VSV-GP vaccine with 6D8 antibody administered on day 5, but there was no difference between the VSV-GP vaccine only and the 13c6 antibody administered on day 5, suggesting the antibodies may impact the vaccination differently to one another.



On day 14, the VSV only and VSV 13c6 d5 groups both had significantly higher IgM titers than the untreated/unvaccinated group, but the VSV vaccine and 13c6 or 6D8 antibodies administered on day -2 or day 0 were not significantly different to the untreated, showing the administration of the 13c6 and 6D8 suppressed the induction of IgM.

By day 21, IgM titers in the VSV-GP vaccinated mice were not significantly different to untreated/unvaccinated, suggesting IgM titers wane in response to VSV vaccination by day 21. However, the VSV 13c6 d5 group have a significantly increased IgM compared to all other vaccinated groups and also the untreated/unvaccinated. This suggests that the administration of the antibody at day 5 may prolong the IgM titers compared to the vaccine only mice. Since the day 14 IgM titers were higher in the VSV 13c6 group than the VSV only, the higher IgM titers at day 21 could also be the result of higher overall IgM titer induction in the VSV 13c6 d5 group.

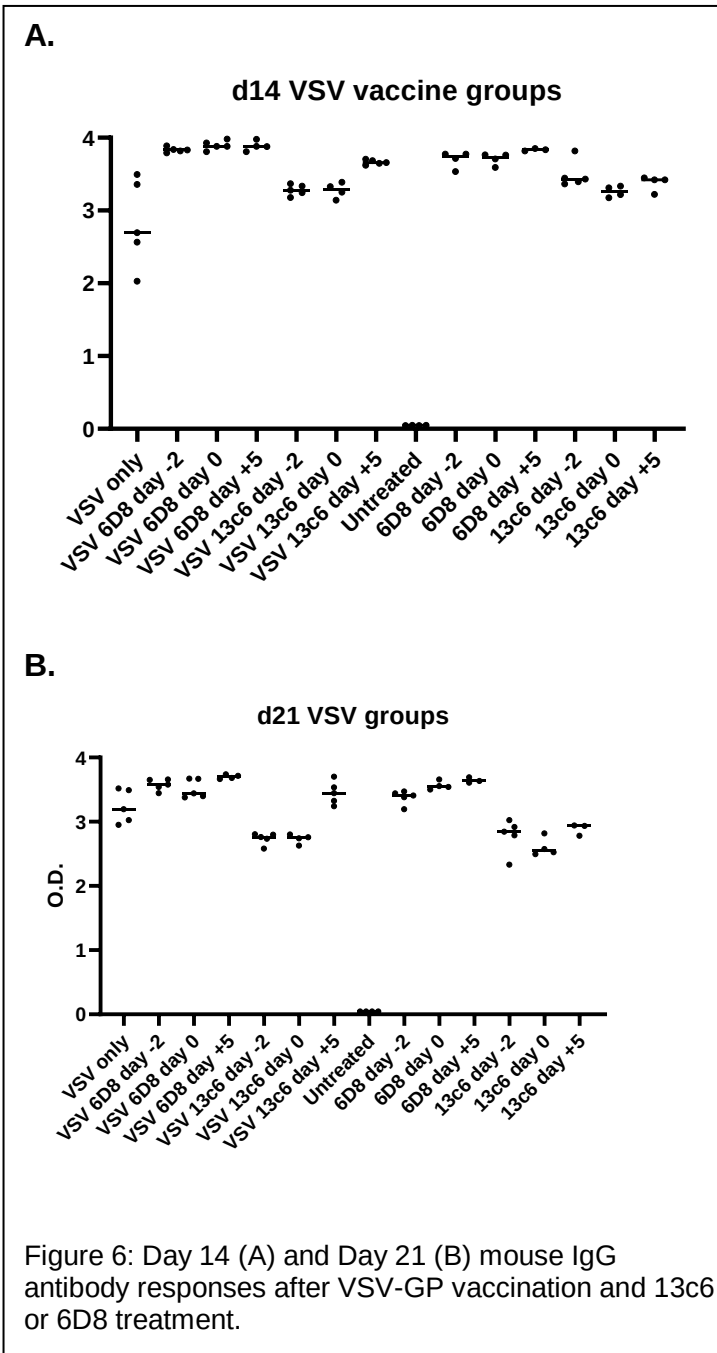


IgG titers after VSV-GP vaccination.

IgG levels were assayed at day 14, 21, 42 and day 63 after mice received VSV-GP on day 0 and were treated with 13c6 or 6D8 on days -2, 0, or 5. Administration of the antibodies 13c6 and 6D8, both murine IgG2a antibodies, in the absence of vaccination, resulted in very high IgG antibody titers in day 14 and 21, and declining but still significant IgG titers at day 42 and 63. The IgG binding had very little background and the untreated/unvaccinated group had very low background absorbance at all time-points.

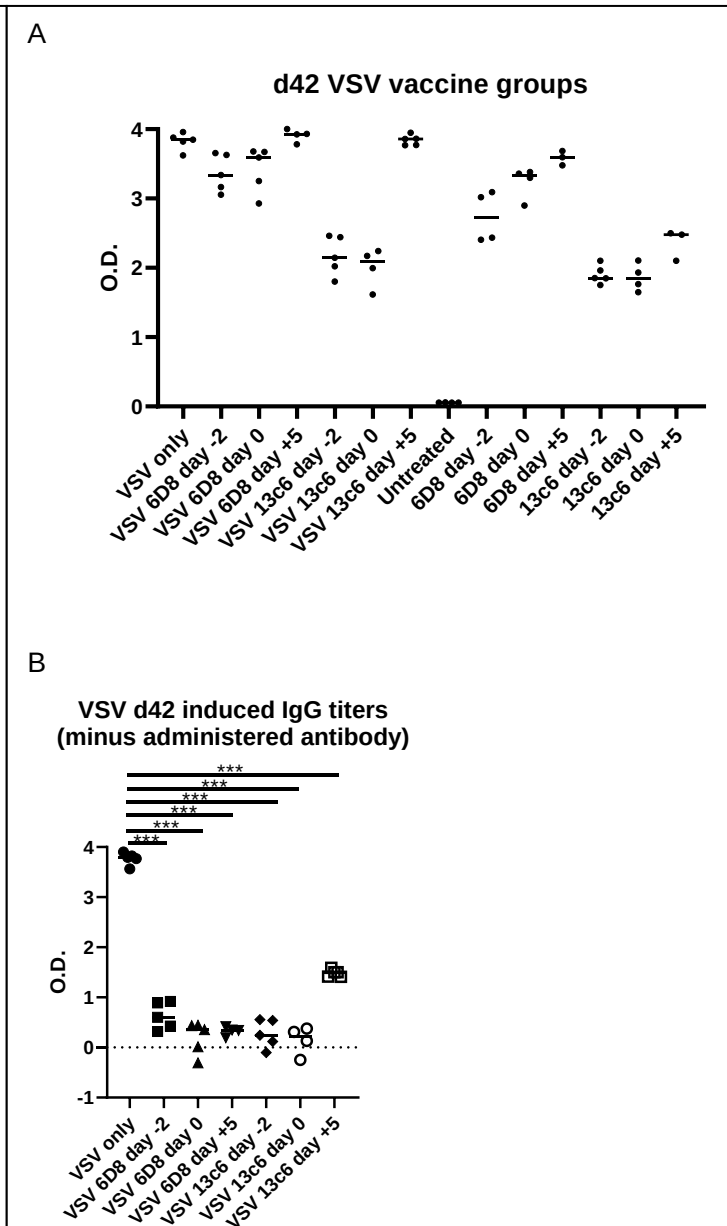
At days 14 and 21, there were very high levels of circulating passively administered 13c6 and 6D8 that prevent differences in any vaccinated groups with the antibodies added from being explored. Figure 6 shows the IgG values for day 14 and 21. Figure 6 shows the increase in concentration of IgG for the VSV-GP only group between panel A and B, at day 21 reaching a similar concentration as the circulating administered antibodies.

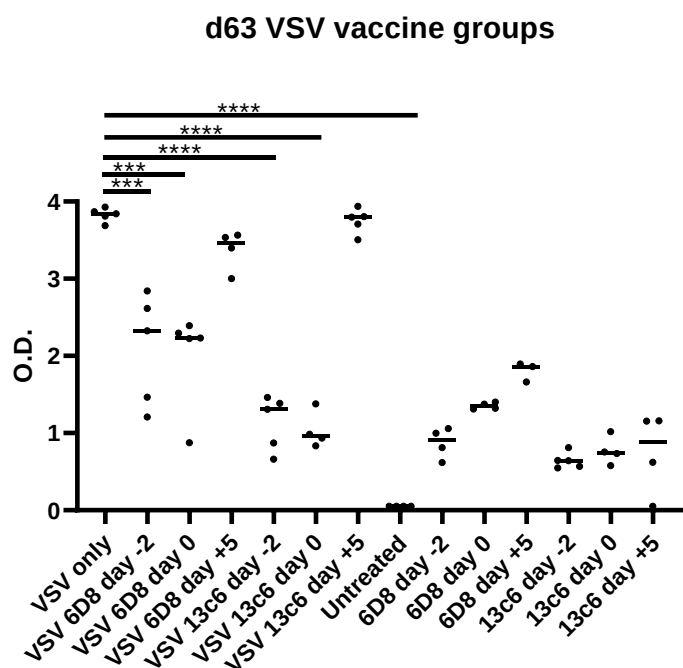
At day 42, there are still significant levels of circulating passively administered 13c6 and 6D8, as shown by the significant difference between IgG titers in the untreated/unvaccinated group and the unvaccinated with antibodies administered on day -2, 0 and 5. Figure 7, panel A shows the day 42 VSV IgG titers. However, by day 42, the VSV-GP vaccine only has induced high IgG titers, and we can compare the IgG levels between the induced and passively administered antibody by subtracting the average antibody only group from their equivalent vaccine + antibody group i.e. IgG titer of VSV-GP with 13c6 day 0 minus the IgG titer of 13c6 day 0 only. The results of this are shown in Figure 7, panel B. It is clear from this that the VSV-GP vaccine has induced strong IgG titers, which are significantly greater than the titers from all the vaccine + antibody groups. However, VSV 13c6 d5 has significantly higher titers than the other antibody groups, suggesting that administering the antibody on day 5 still allows for strong induction of IgG responses. By contrast, the antibody levels of VSV 13c6 d-2 and VSV 13c6 d0 are equal to the antibody levels of 13c6 d-2 and 13c6 d0, suggesting the administration of 13c6 prior to or concurrent with vaccination prevents induction of strong IgG titers. This is also true for all timepoints of 6D8, suggesting the two antibodies behave differently, with 6D8 suppressing IgG responses even when administered at day 5 post-vaccination.



At day 63, administration of 13c6 or 6D8 on days -2 or 0 significantly diminished the mouse anti-GP IgG responses. However, administration of 13c6 or 6D8 on day 5 resulted in significantly higher mouse IgG responses to VSV-GP vaccination, comparable to the VSV-GP only group (Figure 8).

We intend to investigate IgG titers further by using IgG1 as a proxy for overall induced IgG levels to avoid confusion with the IgG antibodies that were passively transferred, which are IgG2a isotype.





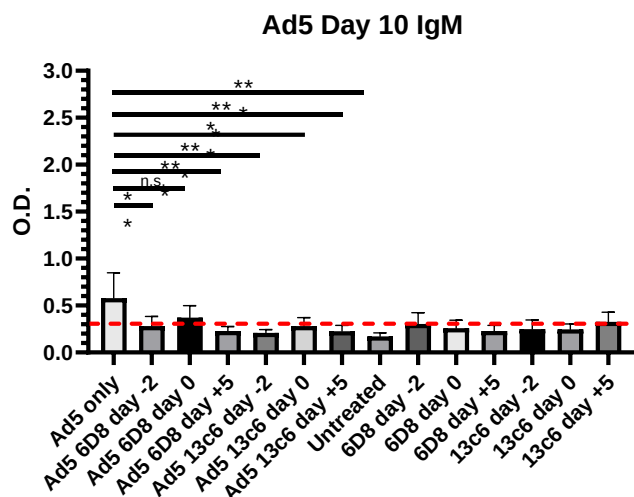
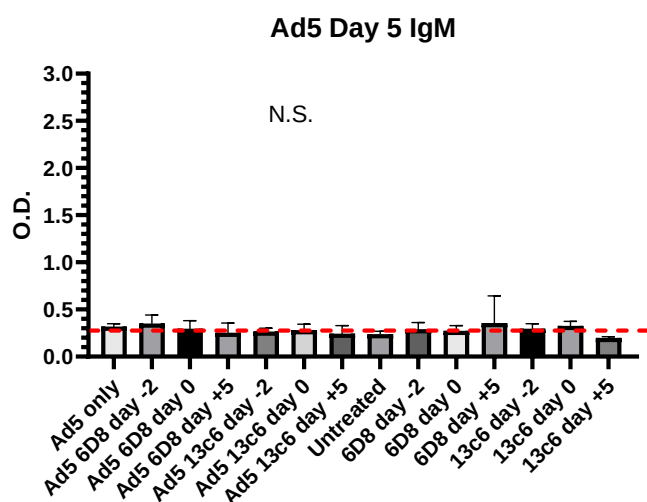
Ad5-GP vaccination.

IgM titers after Ad5-GP vaccination.

Mice receiving Ad5-GP on day 0 and treated with 13c6 or 6D8 on days -2, 0, or 5 were bled on days 5, 10, 14 and 21. Overall, Ad5-GP was weaker at inducing IgM titers than VSV-GP (Figures 2-5 and Figures 9-12). On day 5, there was no induction of IgM in any of the groups with or without the antibody co-administration (Figure 9).

At day 10, significant IgM titers were present in the Ad5 vaccine only group compared to the untreated/unvaccinated group. Administration of 13c6 and 6D8 on days -2 and 5 significantly diminished the mouse IgM anti-GP responses. The Ad5 6D8 d0 is not significantly different from the Ad5 only, but the overall level of induced IgM in both groups is low (Figure

10).



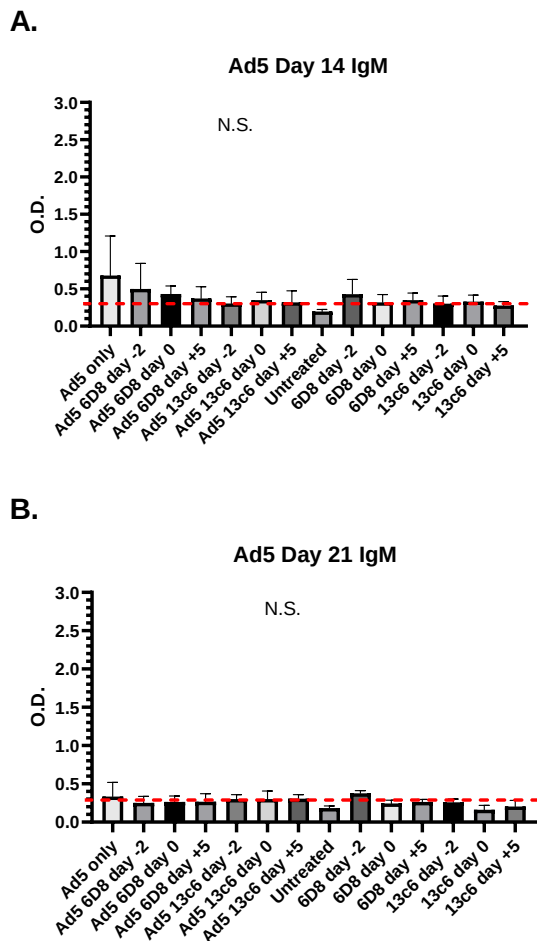


Figure 11: Day 14 (A) and Day 21 (B) mouse IgM antibody responses after Ad5-GP vaccination and 13c6 or 6D8 treatment. N.S. – not significant. The red line indicates the average IgM titer of the unvaccinated mice for each ELISA.

By day 14 and day 21, IgM titers in the vaccinated mice were not significantly different to unvaccinated, suggesting IgM titers wane in response to Ad5 vaccination by day 14. Since several of the VSV vaccinated groups had IgM titers at day 14, this demonstrates the Ad5 vaccine is a weaker inducer of IgM titers.

IgG titers after Ad5-GP vaccination.

IgG levels were assayed at day 14, 21, 42 and day 63 after mice received Ad5-GP on day 0 and treated with 13c6 or 6D8 on days -2, 0, or 5. Administration of the antibodies 13c6 and 6D8, both murine IgG2a antibodies, resulted in very high IgG antibody titers in day 14, 21 and 42, although by day 63 the administered antibodies in the Ad5 vaccine groups appeared to have decreased such that vaccine-induced antibody titers were observable. At day 14 and day 21, the IgG levels in all the vaccine+antibody groups are very high, and the IgG levels in the Ad5 only group are not significantly above the untreated/unvaccinated group. A single mouse from the Ad5 group has higher IgG antibody responses than the rest of the group at each time-point.

At day 42, there are still significant levels of circulating passively administered 13c6 and 6D8, as shown by the significant difference between IgG titers in the untreated/unvaccinated group and the unvaccinated with antibodies administered on day -2, 0 and 5 in Figure 13. As the antibody responses of the Ad5 vaccine are weaker than the VSV-GP vaccine (vaccine only points in Figure 12, 13 and 14 compared to the VSV-GP Figures 6, 7 and 8), the

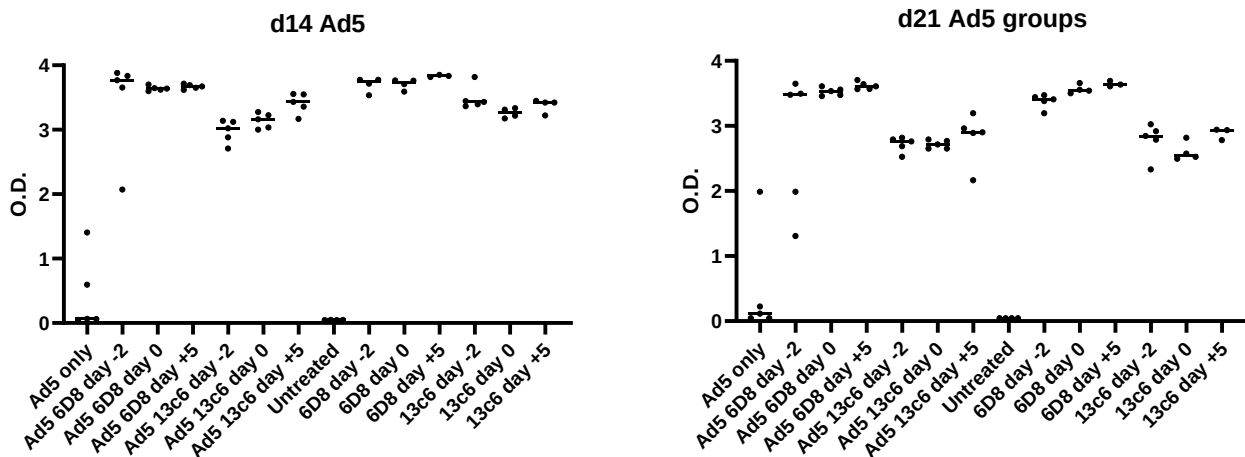
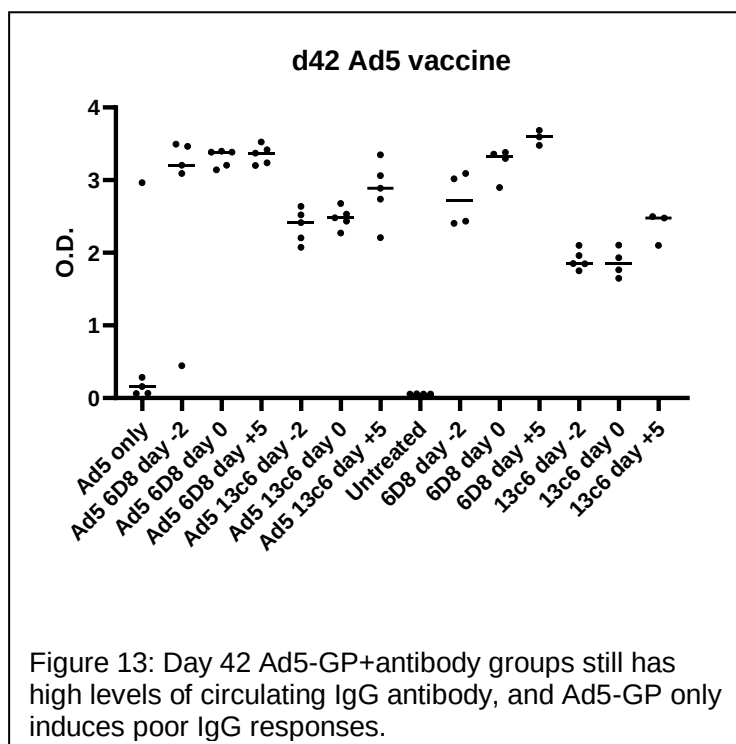


Figure 12: Day 14 (left) and Day 21 (right) mouse IgG antibody responses after Ad5-GP vaccination and 13c6 or 6D8 treatment. Ad5 only vaccine does not induce a strong IgG response at day 14 or day 21, and there are high levels of circulating administered antibodies.



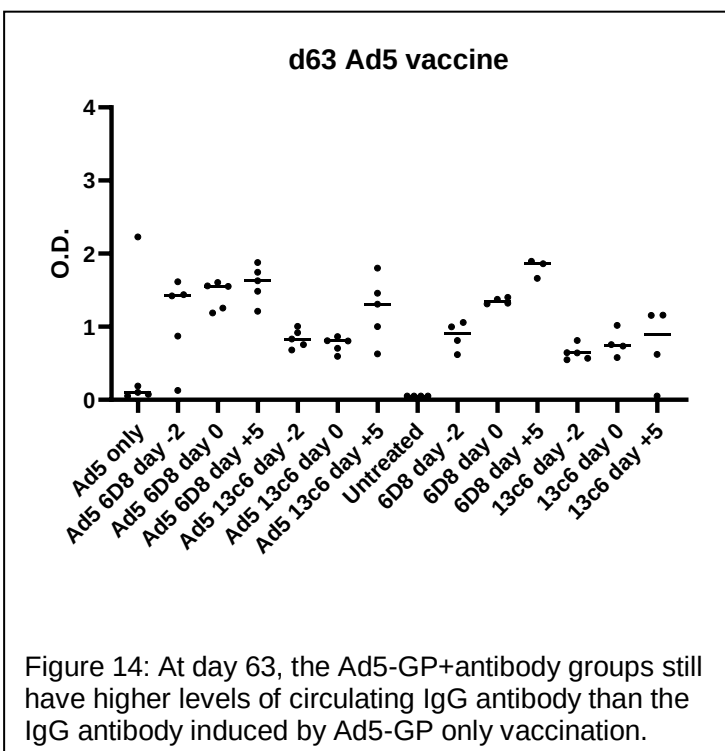
difference between the Ad5 only and untreated/unvaccinated is not large enough to be able to subtract the O.D.s from the antibody only groups from the vaccine+antibody groups as we have for the VSV vaccine to compare induced IgG responses

Despite the fact that by day 63 the circulating 13c6 and 6D8 are lower, the antibody only and the vaccine+ antibody groups still have IgG at comparable concentrations, and the difference between the untreated/unvaccinated and the Ad5 only IgG is still very small (Figure 14). The lack of induction of IgG by Ad5 vaccine was also seen in year 1 in experiments with neutralizing antibodies. We hope that assaying for the IgG1 isotype, as discussed above, will allow for differences in IgG induction between the Ad5 and the Ad5+antibody groups to be more clearly examined.

IgM titers against EBOV-GPdMuc.

EBOV-neutralizing and/or protective monoclonal antibodies, such as KZ52 and the MAb cocktails

ZMAb, ZMapp and MB-003, have been shown to recognize epitopes within or flanking the mucin-like domain. There is evidence that different doses of the VSV-GP vaccine in humans alter the sites of antibody epitopes, including lower (20×10^6 PFU) vaccination having less mucin-like domain epitopes than higher (100×10^6 PFU) (Khurana et al, 2016). The same paper demonstrated that IgM antibodies contribute significantly to neutralizing titers after VSV-GP vaccination in humans. To investigate whether the IgM antibodies were specific to the mucin-like domain, we used Ebola GP without the mucin-like domain protein as an ELISA target for IgM ELISAs. While the IgM ELISAs with different targets are not directly comparable without an IgM antibody standard, it is interesting that the loss of the mucin-like domain did not result in an obvious loss of IgM binding for any of the groups (Figure 15). At day 14 in both the Ebola GP and Ebola GPdMuc ELISAs, both VSV and Ad5 vaccine-only groups are significantly different to the untreated/unvaccinated. However, VSV 13c6 day 5 is significantly increased compared to untreated/unvaccinated in ELISAs against Ebola GP but not Ebola GPdMuc, and VSV 6D8 d-2 and VSV 6D8 d5 are significantly increased compared to untreated/unvaccinated in ELISAs against Ebola GPdMuc but not Ebola GP. This suggests that the addition of the administered antibodies may alter the epitopes of induced IgM.



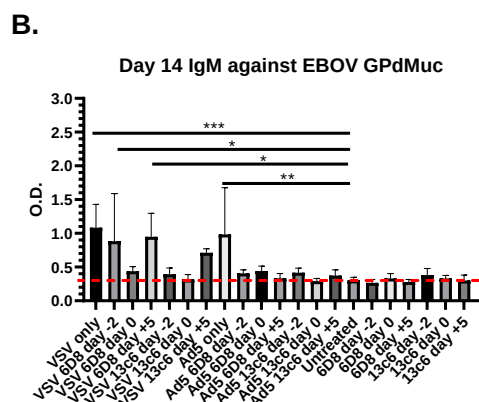
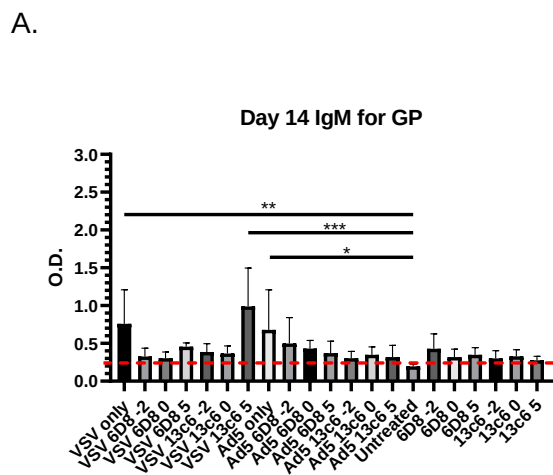


Figure 15. Day 14 IgM in all groups against EBOV GP ELISA target (A), or against EBOV GPdMuc (Ebola GP with the mucin-like domain deleted). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. The red line indicates the average IgM titer of the unvaccinated mice for each ELISA, the background level of binding.

Conclusions (both positive and negative)

- 1) In VSV-GP vaccinated mice, there was a decrease in mouse anti-Ebola virus GP IgM antibody levels on day 10 and day 14 when 6D8 or 13c6 was administered on day -2 or day 0. IgM levels had declined by d21 such that the VSV-GP vaccine was no longer had significant IgM titers.
- 2) In VSV-GP vaccinated mice, vaccine-induced IgG titers were observable at day 42 and day 63, where 6D8 or 13c6 administered on day -2 or day 0 suppressed vaccine-IgG induction compared to VSV-GP only vaccination.
- 3) In VSV-GP vaccinated mice, 13c6 administration at day 5 did not significantly suppress IgG induction, but 6D8 administration at day 5 did suppress IgG induction, suggesting the antibodies interact with the vaccine differently or have different kinetics.
- 4) In Ad5-GP vaccinated mice, significant IgM titers were present in the Ad5-only group at day 14, but not any Ad5 +antibody groups.
- 5) In Ad5-GP vaccinated mice, IgG titers were too low to be allow for comparison between vaccine and vaccine+ antibody groups, as the differences are obscured by the circulating IgG antibodies administered.

d) other achievements.

Stated Goals not met.

- *Live virus challenge.* We were unable to conduct live Ebola virus challenge in our collaborators' BSL-4 facility due to this facility not being able to accommodate these requests because of the COVID-19 pandemic. We are hoping to conduct these experiments in Year 3 as described in section 5.
- *T cell analysis.* We have not yet conducted the day 63 T cell analysis. This work will be accomplished in the coming weeks. We have the splenocytes harvested and frozen for GP epitope responses. We have set up an improved T cell analysis protocol as shown in Figure 23.
- *sGP.* We were not able to acquire sufficient amounts of anti-sGP antibodies for the *in vivo* testing. This is addressed in section 5.

What opportunities for training and professional development has the project provided?

- Student training: Elizabeth Clarke, Tonilynn Baranowski, Julianne Peabody, Sam Goodfellow, and Mahgol Behnia. This project has been used to fund the training of three PhD students in the Bradfute laboratory. Ms. Clarke has participated in the mouse injection, harvesting, and analysis aspects of the work as well as the antibody testing. Ms. Clarke obtained her PhD in January of 2021, and has stayed in the lab on a short-term postdoctoral fellowship. Ms. Clarke is still active in this project. Ms. Baranowski has helped set up the flow cytometric analysis for the T cell responses and is involved in the animal injection and handling aspects of the project. Ms. Behnia has been involved in mouse handling and tissue processing. Ms. Peabody and Mr. Goodfellow have assisted in injections, bleeds, and tissue processing. Together, this grant has significantly impacted the technical and scientific training of these four PhD students and one postdoctoral fellow.
- Research scientist training: Chunyan Ye and Robert Nofchissey. These two individuals are senior research scientists in the Bradfute laboratory with a long history of virus research. Ms. Ye is heavily involved in the injection, tissue processing, and antibody tests reported here. Mr. Nofchissey participated in the mouse injection and tissue harvesting, as well as in the overall setup and organization of the experiments.
- **How were the results disseminated to communities of interest?**
 - Nothing to report. This is due to the cancelation of multiple scientific meetings.
- **What do you plan to do during the next reporting period to accomplish the goals?**
 - We plan to complete the Year 3 goals in the next reporting period.
 - Year 3 contains the major goal of “Comparison of Remdesivir and Favipiravir administration on vaccination.” The subtasks are 1) Determine effects of Remdesivir on vaccine immunogenicity; 2) Determine effects of Favipiravir on vaccine immunogenicity; and 3) Assessment of protection against live virus challenge. We plan to complete these tasks, as we already have the vaccines in hand and the compounds are readily available.
 - We plan to complete the Year 2 goals that were not met in Year 3. This includes live virus challenge studies, T cell analysis, and sGP studies that were postponed due to COVID-19 limitations or product acquisition issues.

4. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
 - Nothing to report. We feel our findings will have an impact but additional experiments are being completed before peer review and publication.
- **What was the impact on other disciplines?**
 - Nothing to report. We feel our findings will have an impact but additional experiments are being completed before peer review and publication.
- **What was the impact on technology transfer?**
 - Nothing to report
- **What was the impact on society beyond science and technology?**
 - Nothing to report

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**
 - Nothing to report
- **Actual or anticipated problems or delays and actions or plans to resolve them**
 - *COVID-19 and personnel limitations.* Due to COVID-19, our institution has limited operations capacity for much of the last year of this grant. Staff capacity was set between 25-50% for most of that time. We have streamlined our large *in vivo* experiments by scheduling multiple personnel on bleed days, analyzing serum at one dilution measured against a monoclonal standard, and cross-training personnel for different kinds of injections. Additionally, our laboratory contributed significantly to SARS-CoV-2 studies since we have access to and training in BSL-3 laboratory work. The Bradfute lab has collaborated with over 35 academic, industry, and government labs to assess neutralizing antibody levels, conduct *in vitro* and *in vivo* drug testing, determine antiviral material testing, and assist in clinical trials for SARS-CoV-2. Although this work has resulted in 10 manuscripts and preprints, it has also limited the work performed on the Year 2 goals when combined with our staffing limits placed by the university. However, we have successfully completed much of the proposed work in Year 2 and hope to complete our pending Year 2 goals in Year 3.
 - *COVID-19 and BSL-4 challenge studies.* Due to the SARS-CoV-2 pandemic, many experiments for this work were adversely affected. The planned BSL-4 live challenge experiments at NIH/NIAID/IRF were not able to be performed, as closures, personnel restrictions, and SARS-CoV-2 work took priority. Our plan to address this issue is to combine the Aim 1 live virus challenge studies (with vaccinated mice injected with monoclonal antibodies) alongside the live virus studies for Aim 2 as well as Aim 3 in Year 3. It is our hope the BSL-4 operations will be available for these studies during that time. Combining the three experiments will also reduce the number of mice to be used, since only one set of controls will be needed.
 - *ChAd3-GP.* Due to restrictions on the availability of ChAd3-GP due to ongoing clinical trials, we had to generate AdV serotype 5-GP for these experiments. There was not a company or academic group we were able to find that would provide the ChAd3 background to construct ChAd3-GP; therefore, we utilized the AdV5 backbone to generate an adenovirus vaccine for these studies. AdV5 was chosen because it was the first adenovirus vaccine to protect non-human primates from Ebola virus infection [4].
 - *Acquisition of sGP antibodies.* We were unable to acquire sufficient amounts of anti-sGP antibodies for the Year 2 experiments. We found anti-EBOV sGP antibody at a cost of \$425 per 100 ug; given that the dose needed was 100 ug/mouse and we would inject 45 mice, the cost was deemed prohibitive. To that end, we are actively assessing other potential source of anti-sGP antibodies for testing in Year 3.
 - *T cell analysis.* We have day 63 splenocytes frozen down from these experiments and will conduct the T cell assays in Year 3. The delay was due to personnel shortages because of COVID-19 restrictions.
- **Changes that had a significant impact on expenditures**
 - Nothing to report
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

- No significant changes. Our IACUC protocol was approved from April 5, 2019 through April 5, 2022.
- **Significant changes in use or care of human subjects**
 - Not applicable
- **Significant changes in use or care of vertebrate animals.**
 - Nothing to report
- **Significant changes in use of biohazards and/or select agents**
 - Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
 - **Journal publications.** Dr. Bradfute has submitted an invited review entitled “Novel drug design strategies for filoviruses” to the journal *Expert Opinion in Drug Discovery*. In it, he discusses the possibility of regimens with both antibodies and vaccines to provide short and long-term protection against EBOV. The manuscript lists this grant as support. This manuscript is currently under review at *Expert Opinion in Drug Discovery*.
 - A manuscript outline is being constructed based on our data, but we are waiting on the live virus challenges prior to submitting a manuscript.
 - **Books or other non-periodical, one-time publications.** Nothing to report.
 - **Other publications, conference papers, and presentations.** Nothing to report.
- **Website(s) or other Internet site(s)**
Nothing to report.
- **Technologies or techniques**
Nothing to report.
- **Inventions, patent applications, and/or licenses**
Nothing to report.
- **Other Products**
Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Name:	Steven Bradfute
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Project Role:	<i>PI</i>
Researcher Identifier (e.g. ORCID ID):	0000-0002-1985-751X
Nearest person month worked:	4
Contribution to Project:	<i>Dr. Bradfute has overseen the project, including experimental design, acquisition of reagents, data analysis, and report generation.</i>
Funding Support:	<i>All of Dr. Bradfute's effort on this project came from this grant.</i>

Name:	<i>Elizabeth Clarke</i>
Project Role:	<i>Graduate Student (through Jan 2021), now postdoctoral fellow</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	<i>Ms. Clarke has overseen the execution of the experiments and participated in injections, tissue harvesting, analysis of antibody levels, and generation of data.</i>
Funding Support:	<i>All of Ms. Clarke's efforts for this project have been funded by this grant.</i>

Name:	<i>Mahgol Behnia</i>
Project Role:	<i>Graduate Student</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	<i>Ms. Behnia has been involved in animal handling, injections, and tissue processing.</i>
Funding Support:	<i>All of Ms. Behnia's efforts for this project have been funded by this grant.</i>

Name:	<i>Tonilynn Baranowski</i>
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Project Role:	<i>Graduate Student</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Ms. Baranowski has been involved in animal handling, injections, and tissue processing.</i>
Funding Support:	<i>DoD</i>

Name:	<i>Julianne Peabody</i>
Project Role:	<i>Graduate Student</i>
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Ms. Peabody has been involved in animal handling, injections, and tissue processing.</i>
Funding Support:	<i>NIH</i>

Name:	<i>Samuel Goodfellow</i>
Project Role:	<i>Graduate Student</i>
Researcher Identifier (e.g. ORCID ID):	0000-0003-0471-8303
Nearest person month worked:	<i>1</i>
Contribution to Project:	<i>Mr. Goodfellow has been involved in animal handling, injections, and tissue processing.</i>
Funding Support:	<i>NIH</i>

Name:	<i>Chunyan Ye</i>
Project Role:	<i>Research Scientist</i>
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	9
Contribution to Project:	<i>Ms. Ye has been involved in animal handling, injections, tissue processing, antibody ELISAs, and neutralizing assays.</i>
Funding Support:	<i>All of Ms. Ye's efforts for this project have been funded by this grant.</i>

Name:	<i>Robert Nofchissey</i>
Project Role:	<i>Research Scientist</i>
Researcher Identifier (e.g. ORCID ID):	0000-0002-7368-6758
Nearest person month worked:	2
Contribution to Project:	<i>Mr. Nofchissey has been involved in animal handling, injections, and tissue processing.</i>
Funding Support:	<i>All of Mr. Nofchissey's efforts for this project have been funded by this grant.</i>

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
 - Nothing to report
- **What other organizations were involved as partners?**
 - **Organization Name:** United States Army Medical Research Institutes of Infectious Diseases (USAMRIID)
 - **Location of Organization:** Fort Detrick, MD, USA
 - **Partner's contribution to the project**
 - **Other.** USAMRIID (Dye lab) supplied the monoclonal antibodies used in this Year 2 report
 - **Organization Name:** Rocky Mountain Labs, NIH/NIAID
 - **Location of Organization:** Hamilton, MT, USA
 - **Partner's contribution to the project**
 - **Other.** RML (Feldmann lab) supplied the VSV-Ebola vaccine used in this Year 2 report

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:** Not applicable.
- **QUAD CHARTS:** Not applicable.

9. **APPENDICES:** Not applicable.

References:

1. Khurana S, Fuentes S, Coyle EM, Ravichandran S, Davey RT, Jr., Beigel JH. Human antibody repertoire after VSV-Ebola vaccination identifies novel targets and virus-neutralizing IgM antibodies. *Nature medicine* 2016; 22:1439-47.