WINONA STATE UNIVERSITY
UNDERGRADUATE STUDENT RESEARCH & CREATIVE PROJECTS
FINAL REPORT

Submit this complete form and a copy of the project end product(s) no later than ten (10) days after the completion of the project. Submit items as email attachments to Grants & Sponsored Projects (grants@winona.edu).

For more information, go to the Student Grants webpage.

Final Report Checklist

The final report **MUST** include this form and the end product (*check to verify inclusion of each component*):

☒ This final report fully completed

☐ A copy of the project end product. Indicate the format of your final product (*select all that apply*):

- ☒ Research Report
- ☐ Presentation
- ☐ Poster
- ☐ Other – Please describe: Click or tap here to enter text.

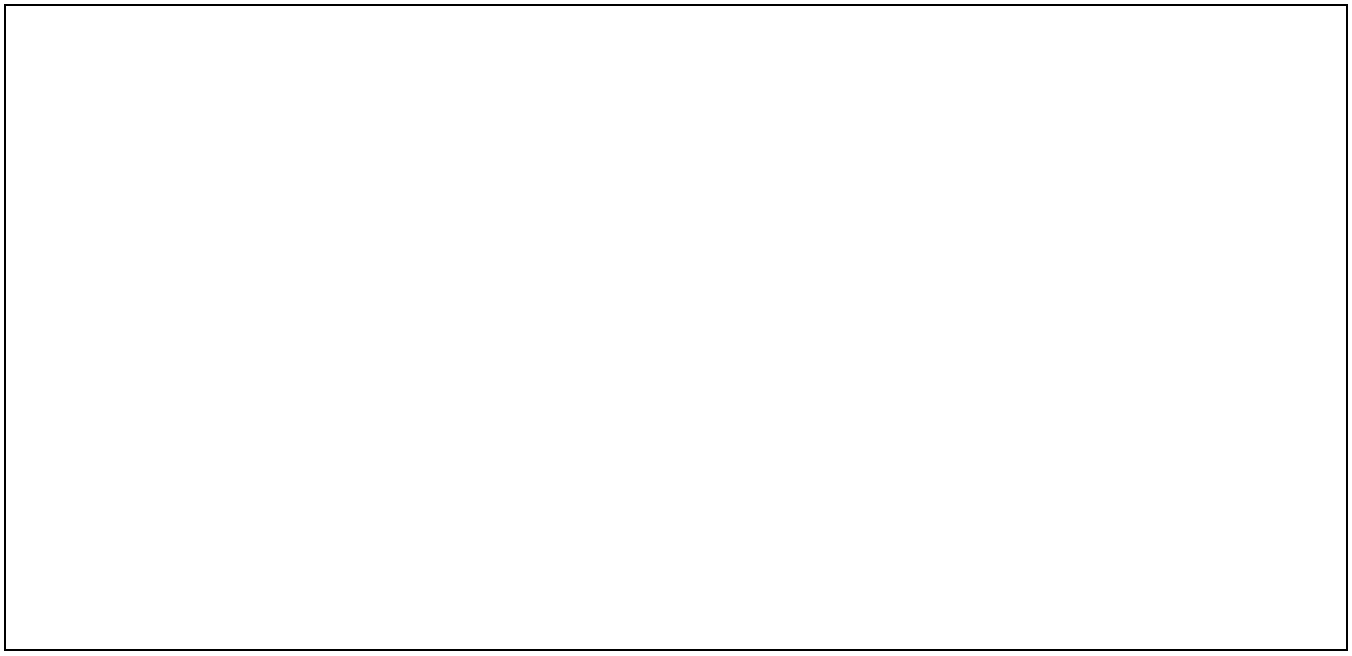
Note: A copy of the project end product will be forwarded to Krueger Library for archival purposes.

Project Information	
Title of Project: Stimulation with Ebola VLPs containing and lacking RIG-I	
Student Name (PI): Julia Fogerty	Student Email: jfogarty16@winona.edu
Co-Investigators Names: Arden Heath, Ramila Shrestha	
Faculty Sponsor: Osvaldo Martinez	Faculty Department: Biology
Was this a capstone, senior thesis, or other degree culminating project? Yes	

Project Abstract

What was the purpose of this research? What were the planned outcomes? What did you do to achieve them? What were the actual outcomes?

Abstract: Ebola virus (EBOV), a filovirus family member, is a highly pathogenic virus that causes Ebola hemorrhagic fever (EHF) resulting in documented mortality rates in humans as high as 50%. Currently, the basic EBOV virus-like particle (VLP) vaccine contains the Ebola virus (EBOV) matrix VP40 and attachment glycoprotein (GP). VLPs are morphologically and biochemically similar to parental virus, yet because they lack a genome and cannot replicate, are safe enough to be used as vaccines. We hypothesize that addition of a constitutionally active retinoic acid-inducible gene 1 (RIG-I) would enhance the ability of the vaccine to induce interferon-dependent immune functions yielding an improved vaccine. Expression of EBOV VP40 in 293T cells induces the spontaneous production of VLPs into the media supernatant and if expressed with EBOV GP, will produce VLPs studded with the attachment GP. Recombinant chimeric constitutively active (ca)RIG-I-VP40 matrix and a nonfunctional mutant L58A (mu)RIG-I-VP40 matrix genes were constructed to produce VLPs containing constitutively active and nonfunctional RIG-I. Supernatant from 293Ts transfected with caRIG-I-VP40, muRIG-I-VP40 or VP40 along with GP expression plasmids were tested for the presence of VLPs. Western blotting of purified VLPs confirmed the presence of RIG-I in caRIG-I-VP40 and muRIG-I-VP40, but not VP40 containing VLPs. Monocyte-like and PMA-differentiated macrophage-like THP-1 Dual cells were treated with nothing, VP40+GP, caRIG-I-VP40+GP, muRIG-I-VP40+GP VLPs as well as LPS and a Vaccinia virus (VACV-70) positive controls and tested for induction of interferon (IFN) signaling. CaRIG-I containing, but not muRIG-I containing VLPs induced interferon signaling from both macrophages and monocytes. The addition of RIG-I to EBOV VLPs revealed an increase of interferon in human peripheral blood monocytes.



Addition of retinoic acid-inducible gene 1 to enhance Ebola virus-like particle vaccine

Julia Fogarty

Department of Biology at Winona State University

Dr. Osvaldo Martinez

2020

Abstract

Ebola virus (EBOV), a filovirus family member, is a highly pathogenic virus that causes Ebola hemorrhagic fever (EHF) resulting in documented mortality rates in humans as high as 50%. Currently, the basic EBOV virus-like particle (VLP) vaccine contains the Ebola virus (EBOV) matrix VP40 and attachment glycoprotein (GP). VLPs are morphologically and biochemically similar to parental virus, yet because they lack a genome and cannot replicate, are safe enough to be used as vaccines. We hypothesize that addition of a constitutionally active retinoic acid-inducible gene 1 (RIG-I) would enhance the ability of the vaccine to induce interferon-dependent immune functions yielding an improved vaccine. Expression of EBOV VP40 in 293T cells induces the spontaneous production of VLPs into the media supernatant and if expressed with EBOV GP, will produce VLPs studded with the attachment GP. Recombinant chimeric constitutively active (ca)RIG-I-VP40 matrix and a nonfunctional mutant L58A (mu)RIG-I-VP40 matrix genes were constructed to produce VLPs containing constitutively active and nonfunctional RIG-I. Supernatant from 293Ts transfected with caRIG-I-VP40, muRIG-I-VP40 or VP40 along with GP expression plasmids were tested for the presence of VLPs. Western blotting of purified VLPs confirmed the presence of RIG-I in caRIG-I-VP40 and muRIG-I-VP40, but not VP40 containing VLPs. Monocyte-like and PMA-differentiated macrophage-like THP-1 Dual cells were treated with nothing, VP40+GP, caRIG-I-VP40+GP, muRIG-I-VP40+GP VLPs as well as LPS and a Vaccinia virus (VACV-70) positive controls and tested for induction of interferon (IFN) signaling. CaRIG-I containing, but not muRIG-I containing VLPs induced interferon signaling from both macrophages and monocytes. The addition of RIG-I to EBOV VLPs revealed an increase of interferon in human peripheral blood monocytes.

Background

The *Ebolavirus* (EBOV) is a deadly virus that is a member of the *Filoviridae* family. Four species of EBOV have been shown to infect and harm humans: Zaire, Sudan, Cote-d'Ivoire, and Bundibugyo.[1] The virus can be spread through direct contact from infected to uninfected individuals, by the handling of contaminated fomites and by the handling of infected dead; EBOV can survive several hours outside of the body and can persist for days after death in infected individuals.[2] Infected EBOV patients may experience hemorrhagic fever that leads to death in up to 90% of documented cases from some outbreaks.[3]

The last three outbreaks of the EBOV have occurred in the last decade (2018, 2017 and 2014), all taking place in West African countries.[4] The outbreak in 2014 spanned six 6 countries totaling 28,600 cases and 11,300 deaths.[4] Since then, research conducted on EBOV vaccines, pathogenesis and therapeutics has increased, however, there is no FDA-approved therapeutic or vaccine for this disease. The most tested EBOV vaccine is based on recombinant Vesicular Stomatitis Virus (VSV-GP) vector.[5]

The VSV-GP recombinant vector vaccine expresses an EBOV protein (in this case glycoprotein (GP) from EBOV) from the vesicular stomatitis virus, which can infrequently cause mild disease in humans.[6] While this vaccine creates epitopes for immune recognition against one antigen (GP), a better vaccine would contain multiple antigens. An epitope is the specific region on a protein antigen that antibodies recognize as foreign, which leads to an immune response. In the past two decades, viral-like particles have shown promising results as vaccines. Viral-like particles may contain proteins that come from the viral matrix of the virus itself. By using multiple viral proteins in a vaccine such as a virus-like particle (VLP) a better immune response may be initiated.[7]

VLP-based vaccines have been shown to be effective and have several advantages as compared to other vaccine types. First, VLP-based vaccines are safer to use (and produce) than attenuated vaccines. While there is a risk of producing revertants when using attenuated vaccines, viral-like proteins-based vaccines are made with an incomplete or no viral genome and therefore cannot replicate to cause disease. In a study with mice, Warfield et. al. showed that VLP-based vaccines were 80-100% effective in protecting mice from mouse-adapted EBOV infection when injected 1-3 days before exposure. VLPs injected into mice also induced high levels of cytokine secretion and immune cells.[8] Antibodies produced from VLP-based vaccines have a higher specificity than antibodies produced from other vaccines due to the multiple viral proteins present.[9] A vaccine is necessary for EBOV because of its high level of virulence.

Part of the effectiveness of EBOV is due to its strategy of disrupting the normal immune response. EBOV, a single-stranded, negative sense RNA virus targets antigen presenting cells (APC), whose functions are essential in stimulating both the innate and adaptive immune response. APCs are white blood cells (dendritic cells, monocytes, etc.) that continuously monitor for foreign invaders and eliminate cellular waste from the body. A specific APC, dendritic cells (DC), are infected and deregulated by EBOV. DCs and other APCs express receptors that recognize, bind and in some cases internalize potentially harmful antigens. Under normal

circumstances, the internalized virus or antigen is broken apart into nonharmful peptides. Surface and internal pattern recognition receptors recognize pathogen antigenic patterns which stimulate the release of cytokines (such as interferon) and the maturation of the DC. The mature DC then brings the antigen to lymphoid tissues where it is presented to T cells. This process activates the adaptive immune response to efficiently remove that foreign invader. In general, viruses counter the activation of the APCs using several different mechanisms to inhibit being detected by the immune system. Two important mechanisms are cytokine downregulation and inhibition of interferon signaling.[4]

It is important to understand the structure of EBOV and the function of the EBOV VP24 and VP35 proteins to acknowledge how EBOV downregulates cytokine and interferon signaling. The EBOV is a filamentous virus that contains 7 structural proteins that partake in the virus' activity in DC. First, entry into the DC is due to the glycoprotein (GP). GP are spike projections that span the enveloped membrane of the virus. It has been shown in previous studies that GP is essential for host cell entry, host tropism and plays a role in viral budding.[3,10] This means GP is required for infection and spread of EBOV from host cell to another uninfected cell. Another structural protein of the EBOV is viral-protein 40 (VP40), which can be found on the inside of the viral envelope.[10] VP40 is an essential protein in EBOV that functions in assembly of the virus and budding of the filamentous virus. The assembly and budding of EBOV is an important part of the spread of the virus from cell to cell.[4,10] VP40 has also been shown to cause immune cell death, especially in T cells.[4] Filamentous VLPs can be produced by expressing GP and VP40 from 293T cells, resulting in the production of stringy VLPs containing GP spike on the VLP surface membrane.[10] Two other EBOV proteins, VP35 and VP24, have been shown to block and inhibit the retinoic acid-inducible gene I (RIG-I).[11] Because VLPs may not always stimulate an immune response efficiently, an adjuvant, RIG-I, will be added to improve the effectiveness of the VLP vaccine.

We hypothesize that a VLP-based vaccine made with GP and a chimeric protein consisting of a constitutively active RIG-I and VP40 will more efficiently and safely stimulate the immune system to prevent EBOV infection. Retinoic acid-inducible gene I (RIG-I), localized in the cytoplasm of the host cells, is a pattern recognition receptor (PRR) specialized in detecting viral RNA (double stranded RNA and single stranded RNA). Type I interferon (IFN) is produced upon detection of viral RNA by RIG-I. IFN is a secreted signaling protein that enhances both the innate and adaptive immune response. We will quantify the effectiveness of adding RIG-I to Ebola VLPs by measuring the levels of IFN produced from monocytes stimulated by 6 different VLPs: (1) VP40 + RIG-I-wildtype + GP, (2) VP40 + mutant-RIG-I + GP, (3) VP40 + RIG-I-wildtype + GPF88A, (4) VP40 + RIG-I-wildtype + GPF88A, (5) VP40 + flagVP40 + GP, and (6) VP40 + VSV-g.[12] EBOV has been shown to interfere with the RIG-I pathway resulting in a decrease of interferon.[13] Human cells infected with modified EBOV VLPs will transduce an adjuvant RIG-I to observe whether the addition of RIG-I enhances the immune response or not. The goal of adding RIG-I to the potential VLP vaccine is to better support immune response through an to increased activity of RIG-I and increase IFN concentrations.

The goal of this experiment is to measure the amount of luminescence using a cell reporter assay equivalent to interferon-containing supernatant produced from human monocytes stimulated with the VP40+GP vs. VP40-RIG-I+GP VLPs to determine if the adjuvant enhances interferon expression.

Materials

Table 1. Mass of DNA (ug) added to each plasmid preparation.

Plasmid	VP40	RIGI-Wt	RIGI M2-1	EBOV GP	GP F88A	Flag VP40	VSV-g
1	4	12		4			
2	4		12	4			
3	4	12			4		
4	4	12			4		
5	4			4		12	
6	10						10

Table 2. Volumes (uL) of solutions added to 10 cm plates

P.	$CaCl_2$	Sterile H_20	HBS
1	144	427	600
2	144	425	600
3	144	415	600
4	144	415	600
5	144	423	600
6	144	418	600

Table 3. Volumes (uL) of VLPs and controls during monocyte stimulation representing equivalent Beta-lactamase units

GPF88A #1	GPF88A #2	Mut RIG I	FLAG VP40	RIG-1 wt / VSV #1	RIG-1 wt / VSV #2	LPS control (1 ug/mL)	VacV control (100ug/mL)
20	19.1	11.5	5.94	14.6	16.61	1	7.5

Methods

Harvesting cells

Human embryonic kidney 293t cells (HEK-293t) were grown in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum. In a sterile hood, cells were washed with ~5 mL of phosphate buffer saline (PBS). After adding PBS, it was quickly aspirated, and ~2 mL of trypsin EDTA was added to each flask then incubated for ~3 minutes at 37 ^{0}C. To halt trypsin activity, ~5 mL of DMEM containing FBS was added to cells. Cells were pipetted up and down repeatedly until no clumps were seen in solution. All cells were harvested and centrifuged for 10 minutes at 1250 rpm. The supernatant was removed, and the cells were re-suspended in 25 mL of DMEM.

Calcium phosphate Transfections

Bacterial-amplified DNA was harvested using MaxiPrep kits (QIAGEN, Germantown, MD), and 175 uL of DNA (VP40 with B-lactamase, RIG-I wild type-VP40 chimera, RIG-I mutant2-1-VP40, EBOV GP, GP F88A, Flag VP40, VSV-g) were transferred to sterile tubes and micro-centrifuged for 2 minutes at 13,000 rpm. After centrifuging 150 uL of the supernatant was removed and placed in new sterile tubes. Twelve 15 mL centrifuge tubes were separated into 2 groups: A and B. Tube A was prepared with 2 M CaCl2, sterile water, and DNA (Table 1). Tube B was prepared with 600 uL 2X Hepes Buffered Saline (HBS). Solution A was then added dropwise over 1.5 min to tube B while bubbling HBS. The new solution was then incubated for 20 min at room temperature. After incubating for 20 min at rm temp, 600 uL of each sample was added to 2 plates each containing ~6.8 million cells per 10 cm plates. VLPs were harvested 3 days later.

Harvesting and enriching for VLPs

Spent supernatant containing secreted VLPs was collected in centrifuge tubes. Cells were pelleted away from the VLP supernatants by centrifuging the supernatant for 8 min at 1100 rpm. Ultracentrifuge tubes were loaded with 5 mL of NTE (100mM NaCL, 10mM Tris, 1 mM EDTA) containing 20% sucrose and then the VLP-containing-supernatant from the previous tubes were overlayered onto the sucrose layer. The samples were then ultracentrifuged using Optima XPN 100 Ultracentrifuge (Beckman Coulter, Indianapolis, Indiana) at 100,000 g for 2 hrs at 10 ^{o}C. Supernatant was aspirated carefully leaving the VLP pellet. The pellet was washed with PBS. The media was aspirated and 100 uL of NTE was added to the pellet. The tubes were put on ice overnight. The next day, left-over VLPs were collected using another 200 uL NTE. Enriched VPLs were placed in 4 ^{0}C refrigerator until further use.

Quantifying VLPs – Beta-lactamase Assay

The VLPs were quantified using a Beta-lactamase assay. A working solution of 3 mL PBS and 3 mL of cytobuster was prepared. The fluorocillin was then diluted to 5 uM using the

PBS/cytobuster solution. To a 96 well, black, clear bottom plate 50 uL of 5uM fluorocillin was added to 12 wells. VLPs (20 uL) were then added in doublet to the prepared wells. Fluorescence spectra was taken after 20 min, 30 min, and 3.5 hrs incubation at room temperature. The data can be seen in Fig. 1.

Isolation of Human Monocytes

MojoSort kit (BioLegend, San Diego, California) was used to isolate human monocytes via negative selection (untouched human monocytes). Two blood samples were collected from anonymous donors. The fresh blood was diluted 1:2 with Hank's Buffered Saline Solution (HBSS). Ficoll-Paque Plus, Amersham Biosciences, Amersham, United Kingdom was then underlaid with the diluted blood and centrifuged at 400 x g for 30 min at 20^0C. The buffy layer was collected and resuspended in HBSS and centrifuged again at 100 x g for 10 min at 20^0C. Cell concentration and viability was determined using a hemocytometer. This pellet was resuspended in 1X MojoSort Buffer. Human TruStain FcX^{TM} was added to cells to block nonspecific antibody binding by incubating cells at rm temp for 10 min. Biotin-Antibody Cocktail was then added to cells and incubated on ice temp 15 min. The cells were resuspended and Streptavidin Nanobeads were added and incubated on ice for another 15 min. The cells were washed with MojoSort^{TM} Buffer and centrifuged at 300 x g for 5 min. Supernatant was discarded and buffer was added. The cell suspension was placed on the magnet for 5 min. The non bound cells were collected. Cells were aliquoted into a 96 well plate at 37^0C with RPMI + 5% human serum with 50,000 cells per well.

Human Monocyte stimulation with VLPS

After cells adhered to the 96 well plate (2 days *can be done in 1 day) media was aspirated and replaced. VLPs and controls were added in triplicate to the plate (Table 3). The plates were then spinoculated for 1 hr at 2000 rpm at 4 ^{0}C. Plates were incubated at 37 ^{0}C. After 24, 48, and 72 hrs, media was collected to test for the presence of interferon and media was replaced.

Results

VLPs were produced in order to stimulate human monocytes. VLPs were produced from human embryonic kidney 293t cells transfected with combinations of DNA as shown in Table 1. The VLPs created were (1) VP40 + RIG-I-wildtype + GP, (2) VP40 + mutant-RIG-I + GP, (3) VP40 + RIG-I-wildtype + GPF88A, (4) VP40 + RIG-I-wildtype + GPF88A, (5) VP40 + flagVP40 + GP, and (6) VP40 + VSV-g (Table 1). A beta-lactamase assay was performed to quantify the relative amounts of each VLP since all VLP preparations were generated with a small amount of beta-lactamase-VP40 which is incorporated into each of the VLP preparations. (Figure 1). A sample of each VLP was incubated with fluorocillin for 20 min, 30 min and 3.5 hrs. The average relative fluorescence unit after 3.5 hrs of incubation for sample were (VP40 + VSV-g) 141,175, (VP40 + RIG-I-wildtype + GP) 124,111.5, (VP40 + mutant-RIG-I + GP) 178,946, (VP40 + RIG-I-wildtype + GPF88A) 103,106.5, (VP40 + RIG-I-wildtype + GPF88A) 107,979.5 and (VP40 + flagVP40 + GP) 346,878.5 respectively (Figure 1). Beta-lactamase is an enzyme that cleaves fluorocillin making it fluorescent. By measuring the fluorescence of fluorocillin, we were able to indirectly measure the relative amount of VLPs. The quantification of VLPs was used to apply the same relative amount of VLPs (beta-lactamase equivalents) to the human monocytes (table 3) in a stimulation assay. Two random samples of human monocytes were isolated using MojoSort kit.

The six VLPs (VP40 + RIG-I-wildtype + GP, VP40 + mutant-RIG-I + GP, VP40 + RIG-I-wildtype + GPF88A, VP40 + RIG-I-wildtype + GPF88A, VP40 + flagVP40 + GP, and VP40 + VSV-g) and 4 controls (background, negative control, LPS and VacV) were added in triplicate to the monocytes and incubated for 72 hrs with supernatant samples taken every 24 hrs. The supernatant was added to THP-1-Dual cells. THP-1-Dual cells contain the enzyme luciferase that is expressed in the presence of interferon. By monitoring the luminescence of luciferase, interferon can be proportionally measured. The luminescence of interferon was analyzed on the 48 hrs sample (Figure 2). The average background luminescence was subtracted from the averages of all the others and graphed with standard deviation (Figure 2). The controls LPS (lipopolysaccharide) and VacV were both known stimulants of interferon (positive controls) while the negative control was not a stimulant of interferon. By measuring the level of interferon produced from each VLP and control, the efficacy of the VLPs to induce interferon can be assayed. The level of interferon was measured in monocyte #1 and #2 after being stimulated by VLPs and controls VP40 + RIG-I-wildtype + GP, VP40 + mutant-RIG-I + GP, VP40 + RIG-I-wildtype + GPF88A, VP40 + RIG-I-wildtype + GPF88A, VP40 + flagVP40 + GP, VP40 + VSV-g, negative control, LPS control, and VacV control were 1,255.7 and 763, 56.7 and 303.3, 37 and 62, 0 and 93.7, 63.3 and 83.7, 1.3 and 167.3, 66 and 0, 121 and 170.7, 214.5 and 0 relative luciferase units, respectively (Figure 2).

Discussion

Quantification using the beta-lactamase assay showed VLP production. It was predicted that the VLPs containing both GP and VP40 should produce the highest amount of VLPs. The EBOV protein GP is required for cellular entry while the EBOV protein VP40 is required for VLP budding from the cell (HEK 293t cells). This was tested by the positive control VP40 + GP + flag-VP40 and the altered GP (GPF88A). The VLP with the highest production was VP40 + GP + flagVP40, which was more than 2 times greater than VP40 + RIG-I-wildtype + GP, VP40 + mutant-RIG-I + GP and VP40 + VSV-g and over 3 times greater than VP40 + RIG-I-wildtype + GPF88A. Comparing the flagVP40 VLP to the other VLPs illustrates the importance of VP40 in viral budding. Higher production of VLPs can be made with VP40. The VLPs that had the lowest production were VP40 + RIG-I-wt + GPF88A (Figure 1). The average amount of VLPs produced from VP40+ RIG-I-wt + GPF88A was 105,543 units of relative fluorescence. The mutated GP VLPs contained a nonfunctional attachment protein and illustrated a reduced amount of VLPs produced. In VP40 + RIG-I-wt + GP had 1.18 times greater VLP production than mutGP, while VP40 + mutRIG-I + GP had 1.22 times more VLP produced. The VSV-g VLP, which did not contain EBOV GP but a different attachment protein, produced 1.34 times more VLPs than mutGP (Figure 1). Although the VSV-g produced more VLPs than the EBOV GP, the EBOV GP is necessary for immune cell recognition of EBOV.

Human monocytes were isolated and stimulated with VP40 + RIG-I-wildtype + GP, VP40 + mutant-RIG-I + GP, VP40 + RIG-I-wildtype + GPF88A, VP40 + RIG-I-wildtype + GPF88A, VP40 + flagVP40 + GP, and VP40 + VSV-g as well as LPS and VacV. While LPS increased IFN in both monocyte harvested from two different individuals (1 and 2), VacV did not have significant IFN stimulation (Figure 2). The insignificant stimulation by the positive controls (LPS and VacV) may be due to the inability to work on the human monocytes prepared. Interferon produced by the monocytes were measured by luminescence. The highest production of interferon was seen in the monocytes stimulated by VP40 + RIG-I-wt + GP. The RIG-I adjuvant's effectiveness can be seen when comparing VP40 + RIG-I-wt + GP with VP40 + mutRIG-I + GP. In monocyte #1, VLP VP40 + RIG-I-wildtype + GP had a 22-fold greater production of IFN than VLP VP40 + mutant-RIG-I + GP; while in monocyte #2, VLP VP40 + RIG-I-wildtype + GP had a 2.5-fold increase in IFN production. Both monocyte #1 and #2 present data that demonstrates RIG-I-wt effectiveness at increasing IFN production. The lowest production of interferon was seen in the monocytes stimulated by the negative control and VLPs VP40 + RIG-I-wt + GPF88A. With the GPF88A mutant glycoprotein, entry into monocytes will be abrogated. The RIG-I pathway of inducing IFN production is an intracellular process. Without GP-mediated entry into the cell, the RIG-I has no access to the cytoplasm to stimulate IFN production. This further emphasizes the importance of GP in the VLP. The samples VP40 + mutRIG-I + GP, VP40 + VSV-g, and LPS all had similar levels of interferon production (Figure 2). These similar levels illustrate that VLPs without the RIG-I adjuvant and functional GP produce similar effects as the known IFN stimulator LPS. LPS and VacV stimulate IFN through inflammation, while the PRR, RIG-I, is directly in the pathway for IFN stimulation, which apparently makes it more effective at producing IFN in monocytes. The adjuvant, RIG-I, showed

better stimulation of human monocytes. To further illustrate the effectiveness of VLPs using VP40 + RIG-I + GP, a study testing interferon production in mice can be done.

References

1. Ramanan, Parameshwaran, Reed S. Shabman, Craig S. Brown, Gaya K. Amarasinghe, Christopher F. Basler, and Daisy W. Leung. “Filoviral Immune Evasion Mechanisms.” Viruses 3, no. 9 (2011): 1634–49. https://doi.org/10.3390/v3091634.

2.“Transmission | Ebola Hemorrhagic Fever | CDC,” November 6, 2019. https://www.cdc.gov/vhf/ebola/transmission/index.html.

3.Martinez, Osvaldo, Joshua Johnson, Balaji Manicassamy, Lijun Rong, Gene G. Olinger, Lisa E. Hensley, and Christopher F. Basler. “Zaire Ebola Virus Entry into Human Dendritic Cells Is Insensitive to Cathepsin L Inhibition.” Cellular Microbiology 12, no. 2 (2010): 148–57. https://doi.org/10.1111/j.1462-5822.2009.01385.x.

4.Pleet, Michelle L., Allison Mathiesen, Catherine DeMarino, Yao A. Akpamagbo, Robert A. Barclay, Angela Schwab, Sergey Iordanskiy, et al. “Ebola VP40 in Exosomes Can Cause Immune Cell Dysfunction.” Frontiers in Microbiology 7 (November 7, 2016). https://doi.org/10.3389/fmicb.2016.01765.

5.Medaglini, Donata, Francesco Santoro, and Claire-Anne Siegrist. “Correlates of Vaccine-Induced Protective Immunity against Ebola Virus Disease.” Seminars in Immunology 39 (2018): 65–72. https://doi.org/10.1016/j.smim.2018.07.003.

6.Bull, James J., Scott L. Nuismer, and Rustom Antia. “Recombinant Vector Vaccine Evolution.” PLOS Computational Biology 15, no. 7 (July 19, 2019): e1006857. https://doi.org/10.1371/journal.pcbi.1006857.

7.Warfield, Kelly L., and M. Javad Aman. “Advances in Virus-Like Particle Vaccines for Filoviruses.” The Journal of Infectious Diseases 204, no. suppl_3 (November 1, 2011): S1053–59. https://doi.org/10.1093/infdis/jir346.

8. Warfield, Kelly L., Jeremy G. Perkins, Dana L. Swenson, Emily M. Deal, Catharine M. Bosio, M. Javad Aman, Wayne M. Yokoyama, Howard A. Young, and Sina Bavari. “Role of Natural Killer Cells in Innate Protection against Lethal Ebola Virus Infection.” The Journal of Experimental Medicine 200, no. 2 (July 19, 2004): 169–79. https://doi.org/10.1084/jem.20032141.

9. Dhama, Kuldeep, Kumaragurubaran Karthik, Rekha Khandia, Sandip Chakraborty, Ashok Munjal, Shyma K. Latheef, Deepak Kumar, et al. “Advances in Designing and Developing Vaccines, Drugs, and Therapies to Counter Ebola Virus.” Frontiers in Immunology 9 (August 10, 2018). https://doi.org/10.3389/fimmu.2018.01803.

10. Noda, Takeshi, Hiroshi Sagara, Emiko Suzuki, Ayato Takada, Hiroshi Kida, and Yoshihiro Kawaoka. “Ebola Virus VP40 Drives the Formation of Virus-Like Filamentous Particles Along with GP.” Journal of Virology 76, no. 10 (May 2002): 4855–65. https://doi.org/10.1128/JVI.76.10.4855-4865.2002.

11.Spiropoulou, Christina F., Priya Ranjan, Melissa B. Pearce, Tara K. Sealy, César G. Albariño, Shivaprakash Gangappa, Takashi Fujita, et al. “RIG-I Activation Inhibits Ebolavirus Replication.” *Virology* 392, no. 1 (September 15, 2009): 11–15. https://doi.org/10.1016/j.virol.2009.06.032.

12.Luo, Dahai. “Toward a Crystal-Clear View of the Viral RNA Sensing and Response by RIG-I-like Receptors.” RNA Biology 11, no. 1 (2014): 25–32. https://doi.org/10.4161/r

13.Valmas, Charalampos, and Christopher F. Basler. “Marburg Virus VP40 Antagonizes Interferon Signaling in a Species-Specific Manner ▿ .” Journal of Virology 85, no. 9 (May 2011): 4309–17. https://doi.org/10.1128/JVI.02575-10.

Figures

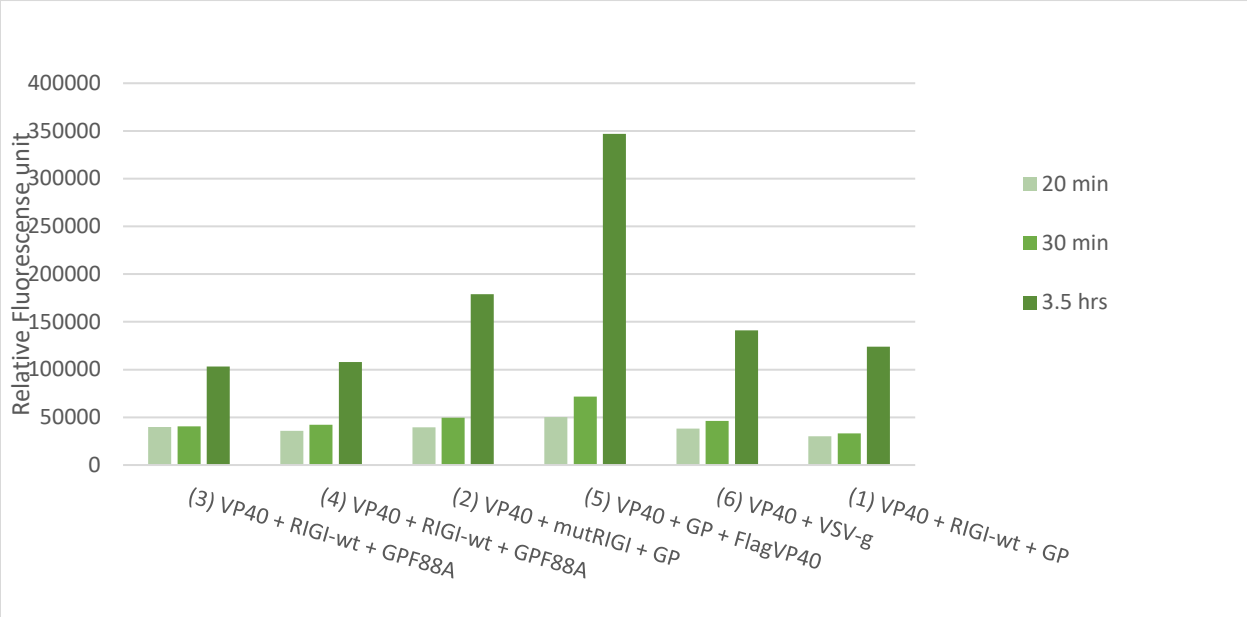


Figure 1. VLP quantification using Beta-lactamase assay and relative fluorescence taken 20 minutes, 30 minutes, and 3.5 hours after incubation with fluorocillin.

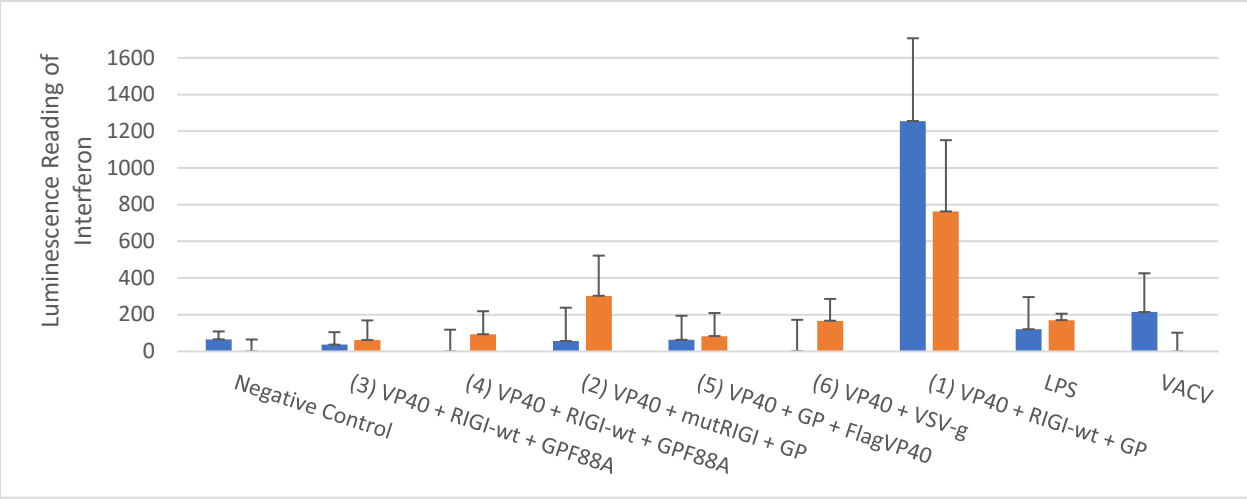


Figure 2. Interferon quantification by luminescence reading of VLP stimulated human monocytes of two individuals (blue and orange).