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Project Information

Title of Project: Stimulation with Ebola VLPs containing and lacking RIG-I	
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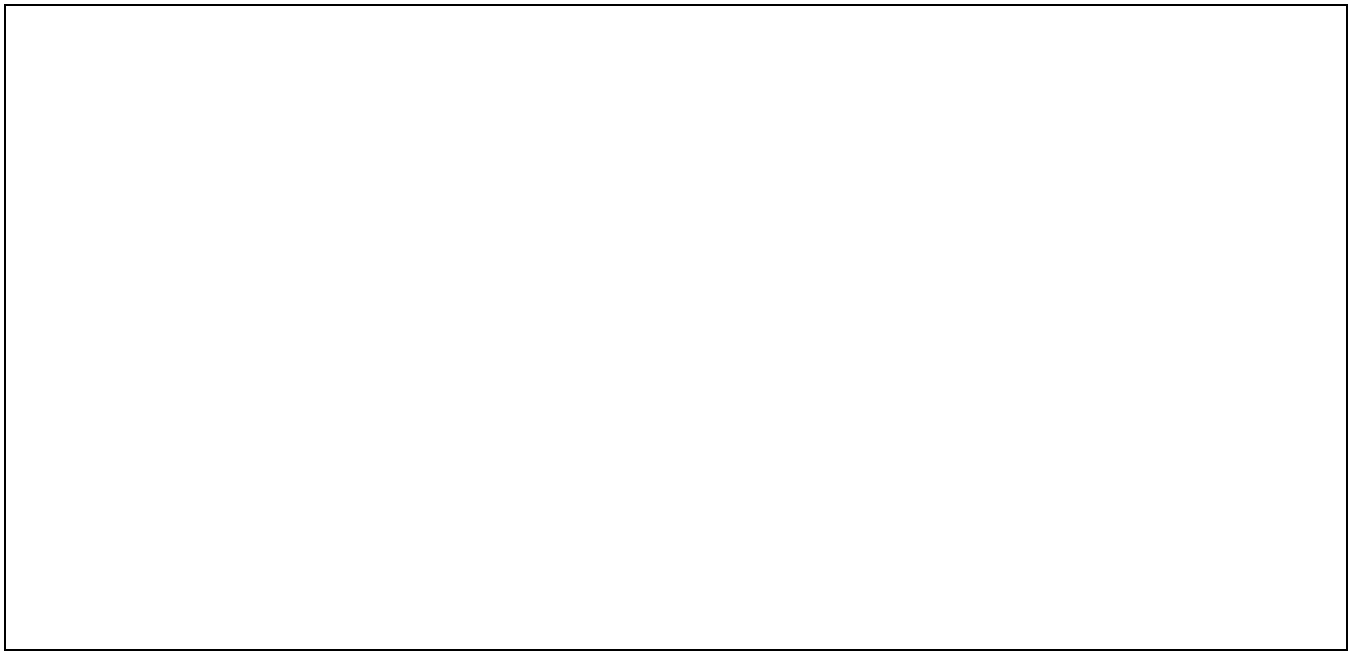
Co-Investigators Names: Ramila Shrestha, Julia Fogerty	
Faculty Sponsor: Osvaldo Martinez	Faculty Department: Biology
Was this a capstone, senior thesis, or other degree culminating project? Yes	

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. These results lead us to conclude that supplemented CaRIG-I would be ideal for robust induction of interferon-dependent immune functions, which may improve vaccine efficacy.



Thesis: Comparative Response of Immune Cell Interferon Production from Ebola Viral Particles.

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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. These results lead us to conclude that supplemented CaRIG-I would be ideal for robust induction of interferon-dependent immune functions, which may improve vaccine efficacy.

Introduction

Ebola Zaire (EBOV) shares the Filoviridae family with four other species of highly virulent Ebolaviruses; Bundibugyo, Sudan, Reston and Tai Forest, based on location of discovery. EBOV is an enveloped, negative-sense RNA virus holding the highest mortality rate ranging 60-90% (Kadanali et. al, 2015). The first recorded case of EBOV occurred in 1976 in sub-Saharan Africa, and the most recent, serious outbreak occurred in 2014 in West Africa which claimed 11,000 lives. Between 1976 and 2014, outbreaks have normally been contained quickly due to effective implementation of public health safety measures to prevent spread. With respect to the 2014 outbreak that had a record number of cases, host disease processes have been more meticulously studied (Ploquin et. al, 2018).

The incubation period for EBOV in humans is approximately 2-21 days, with the virus being detectable in blood only during acute infection. Acute infections can initially be mild and present with fever, myalgias, malaise, gastrointestinal issues, and maculopapular rash (Kadanali et. al, 2015). Lethal EBOV infections involve internal and external hemorrhaging, secondary to coagulopathy, with more than half of affected patients suffering from severe systemic viremia and multisystem organ failure. The extent of viremia was a strong predictor of fatality and was closely monitored in some treatment settings throughout hospital admission (Faye et. al, 2015). EBOV outbreaks initially are a result of zoonotic transmission or handling of infected animals and their body fluids. Human-to-human transmission occurs primarily through handling of infectious body fluids during care or death investigation, rituals, and laboratory exposures. This virus has been classified as a Biosafety Level (BSL)-4 pathogen, requiring stringent personal protective measures and protocol for study, due to lethality and lack of treatment options (Martines et. al, 2015).

Antigen Presenting Cells (APCs) including monocytes, macrophages, and dendritic cells (DCs) are exceptionally susceptible to EBOV infection and are sites of viral amplification. Undifferentiated monocytes are more restrictive, but the addition of EBOV to monocytes induces differentiation into macrophages (Martinez et. al, 2013). EBOV moves beyond the site of infection, via blood and lymphatic systems, to infect organs like the liver and kidneys along with regional lymphatic organs. This access to cells of the mononuclear phagocytic system removes the need to infiltrate tissue or cell barriers of target organ tissues (Martines et. al, 2015).

EBOV expresses at least eight proteins; the nucleoprotein (NP), viral protein (VP) 35, VP40, glycoprotein (GP), VP30, VP24, and polymerase (L). The viral GP binds with several target host cell surface proteins including non-specific C-Type lectins (CLECs) and Phosphatidylserine (PtdSer) receptors) to mediate entry (Yu et. al, 2017). CLECs and PtdSer receptors collectively represent at least 8 recognition domains, 2 protein complexes, and a family of 3 receptor tyrosine kinases which facilitate entry and attachment events (Moller-Tank & Maury, 2015). The outer envelope of the virus contains a trimer of GP, a class I fusion protein composed of covalently linked processed GP products, GP1 and GP2. GP1 being the receptor binding subunit, and GP2 being the fusion subunit. After initial internalization, or macropinocytosis of EBOV, cathepsins B & L process GP1 in the early and late endosome to allow GP1 to interact with host protein Niemann-Pick C1 (NPC-1) and GP2 to fuse to the endosomal membrane (Salata et. al, 2019). Specifically, GP2 fusion loop forms a transmembrane

bundle containing two heptad repeat domains, leading to fusion of host and viral membranes allowing the viral RNA to exit the endosome and be released into the cytoplasm for replication (Yu et. al, 2017).

The cytoplasm of host cells is home to cytoplasmic pattern-recognition receptors (PRRs) that are important for activation of immune responses against intracellular pathogens. The PRR Retinoic Acid Inducible Gene-I (RIG-I) activates type I interferon production when viral genetic material, specifically 5'PPP-ssRNA or dsRNA is sensed. Viral replication of EBOV, and other negative-sense RNA viruses like vesicular stomatitis, is inhibited upon the activation of RIG-I (Spiropoulou et. al, 2009). Initial viral replication induces RIG-I and/or MDA5 to activate serine/threonine kinases to phosphorylate IRF-3 and 7. This promotes IFN-A/B gene expression for the paracrine production of type 1 interferon to signal the presence of virus infection. EBOV VP35 inhibits interferon production (Messaoudi et. al, 2015).

EBOV Virus-Like Particles (VLPs) are induced when VP40 is expressed in a cell. VLPs are similar to the parent virus that bud from the membrane of infected cells. These VLPs lack the genes necessary for replication, virulence, and pathogenicity; making them safe for study outside of BSL-4 laboratories (Marcinkiewicz et. al, 2014). Budding of the VLPs takes place in the intracellular membrane, plasma membrane, or multivesicular bodies. VLPs housing VP40, GP, and NP can be used as a vaccine and show a protective role in experimental animal models of EBOV infection (Falasca et. al, 2015).

VLPs have shown promise as novel vaccines. VLPs pose less risk than attenuated viral vaccines that may revert to wild type. They are able to produce a response in both cell-mediated and humoral immune responses. For example, the vaccine against human papilloma virus (HPV) is a VLP vaccine (Fuenmayor et. al, 2017). In another study, vaccinating mice with EBOV VLPs exhibited increases in CD40, 80, and 86 with MHC Class I & II, and increased secretion of IL-6, IL-10 and macrophage inflammatory protein (MIP)-1a, all signs of immune activation. Cell mediated immunity was also demonstrated in the activation of $CD19^+$ B-Cells, along with $CD4^+$ and $CD8^+$ T-Cells. Furthermore, anti-EBOV antibodies induced by VLPs provided protection from lethal EBOV challenge in mice (Warfield et. al, 2003).

Adjuvants are used frequently in combination with antigens in vaccines to safely induce a stronger immune response in the recipient. Some of the most common adjuvants used include mineral salts, microparticles, microbial products, and emulsions. These adjuvants are noted to promote sustained antigen release and cellular recruitment at injection site, increase antigen uptake and presentation to APCs, and up-regulate production of cell signaling molecules (Awate et. al, 2013). One example includes the addition of a dsRNA-like adjuvant called polyinosinic-polycytidylic acid (poly-IC) or its derivative, poly-IC poly-L-lysine carboxymethylcellulose (poly-ICLC). Poly-IC as an adjuvant proven to be effective at inducing anti-EBOV antibodies for immunity, but protection isn't entirely dependent on anti-EBOV antibodies. Antibody-independent B-Cell protection mechanisms still requires further investigation (Cooper et. al, 2017).

Another type of vaccine candidate for EBOV is a live-attenuated recombinant vesicular stomatitis virus that expresses the EBOV GP. Also called V920, this vaccine was tested and

approved by the FDA and named ERVEBO (Eisele, 2019). It was uniquely stable in conditions that would render other vaccines and its components unusable in the field within low-income countries with little access to proper storage. It provided generally effective protection for at least two years and yielded a good risk-benefit analysis. Although this protection tapered, there are some clinically significant adverse effects including, but not limited to; fever, myalgia/arthralgia, arthritis, headaches, thrombocytopenia, leukopenia, lymphopenia, monocytosis and increases in liver enzymes. Along with the adverse side effects being investigated, children exhibited high levels of rVSV in the plasma and viremia beyond a week post-vaccination with viral shedding in urine and saliva (Bache et. al, 2020). An additional study using a similar rVSV-EBOV vaccine with a smaller sample size indicated the need for a second dose. The second dose yielded increased antibody titers at almost 2 months and reduced adverse effects, but the protection from the antibodies diminished after 6 months (Regules et. al, 2015).

The purpose of this study is to determine whether addition of a constitutively active RIG-I to Ebola VLPs can stimulate type I interferon production from treated human blood peripheral monocytes

Materials & Methods

Plasmids

We transformed competent cells with vectors expressing six different EBOV proteins. The viral proteins include EBOV GP, GPF88A (Mutant on Gene 88, single base, Phenylalanine to Argenine), RIG-I Wild Type and Mutant (L58A), Recombinant Vesicular Stomatitis Virus (r-VSV), Flag VP40, and VP40 B-Lactamase.

Cell Preparation and Maintenance

293T Cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum, and APS (Amphotericin B, Penicillin, and Streptomycin) solution. Cells were incubated at 37°C under 5% CO_2.

Maxi-Preparations of Expression Vectors

Maxi-Preparations were performed using the Plasmid Plus Maxi Kit (QIAGEN, Germantown, MD)to purify plasmid DNA per manufacturer instructions, using DNA.

Calcium Phosphate Transfection of Expression Vectors

Performed Day 1 of Calcium Phosphate Transfection Protocol from the Calcium Phosphate Transfection Kit for a 100mm dish (Invitrogen, Carlsbad, CA)

In summary, two solutions were prepared and used to create a mixture to add to 293T cells. Solution A containing purified, sterile DNA was mixed with multiple plasmids at a ratio of 3:1:1 for the first 5 plasmid mixtures, and 1:1 for the last plasmid mixture (see table 1). Solution A contains DNA mixture, sterile H_2O, and 1M $CaCl_2$ (Table 1). Solution B solely contained 2X Hepes Buffered Saline (HBS). Solution A was slowly added to solution B over the course of two minutes and incubated at room temperature for 20 minutes, and then added dropwise to cells in two separate 10cm dishes. Transfected cells incubated for 72 hours at 37°C under 5% CO_2.

VLP Harvest and Purification

All work with VLPs was completed in a sterile hood to maintain consistent aseptic technique. Cells were pelleted out of harvested supernatant continaing VLPs before layering onto a sucrose cushion to enrich for VLPs.

Purification of the VLPs consisted of two cycles of ultracentrifugation at 100,000Gs for 2.5 hours at 10°C through a 20% sucrose cushion. Supernatant was aspirated after each spin, but PBS was carefully added after the first cycle, and removing the remaining sucrose cushion with the supernatant occurred at the end of the second spin. The pellet consisted of VLPs and was resuspended in 100uL of NTE buffer (filtered through a 0.2 micron filter to ensure sterility). Samples incubated on ice overnight sealed with parafilm, then were moved to microcentrifuge tubes.

Quantification of VLPs- β-Lactamase Assay

Quantification of VLPs was measured using relative fluorescence in a black microplate using Molecular Devices Spectramax i3x spectrophotometer (Company name, central office city, state) and was analyzed with SoftMax Pro software. The plate was bottom-read at a wavelength of 535 nm, 495 nm excitation, and medium shake for 5 seconds before the first read at each time interval.

The stock solution used to fill the microplate for each sample of VLP consisted of 1mL PBS (with Mg^{2+} and Ca^{2+})/Cytobuster, 10uL of fluorocillin, and 20uL of assigned VLP. 50uL of the solution was added to each well in duplicate for each of the 6 VLPs, were sealed in parafilm and covered for darkness, to incubate at room temperature (25°C). The plate was read at time intervals of 20 minutes, 30 minutes, and 3.5 hours (Figure 1).

Monocyte Isolation

Four anonymous donors (A, B, Y, and Z) gave 28mL of Whole Human Blood each and was collected in standard 4mL EDTA (Purple Top) Vacutainer Tubes. Y and Z were harvested first, then A and B were harvested in a repeat of this protocol. Each subject's blood products were placed into designated 50mL tornado tubes (Red Tops), and continually mixed to prevent coagulation. Blood was diluted 1:2 with Hank's Buffered Saline Solution (HBSS) in preparation for monocyte isolation.

Initial isolation of monocytes and removal of platelets were completed by following the GE Healthcare Ficoll-Paque PLUS protocol per manufacturer instructions with the following modifications. The pellet of PBMCs were resuspended for monocyte isolation.

The BioLegend MojoSort™ Human Pan Monocyte Isolation Kit (Company name, central office city, state) was used, per manufacturer instructions, with the following modifications. The Platelet Removal Procedure was not performed. We analyzed cell viability and quantity of viable cells with the ViaCell XR Cell Counter prior to adding Human TruStain FcX™ (Fc Receptor Blocking Solution), Biotin Antibody Cocktail, and the magnetic Streptavidin Nanobeads. To perform this, the pellet from samples Y and Z in the Ficoll-Paque PLUS protocol were resuspended in 10.5mL of MojoSort Buffer provided in the kit. The pellets from samples A and B in the second round of enacting this protocol were resuspended in 11mL of MojoSort™ buffer. 0.5mL of resuspended pellet samples Y and Z were diluted 1:2 with HBSS for cell counting, while samples A and B were not diluted and solely used resuspended product.

The volume of Human TruStain FcX™ (Fc Receptor Blocking Solution), Biotin Antibody Cocktail, and the magnetic Streptavidin Nanobeads used in this protocol based on the Total Viable Cells count on the cell counter with confirmation by manual hemocytometry (Table 2). Incubation times, centrifugation, magnet use for liquid fractions for gathering unlabeled cells remained according to protocol.

The fractions are quantified for viable cells using 0.5mL of cells and 0.5mL of MojoSort™ buffer in the ViaCell XR after aforementioned protocol was completed.

Monocyte Culturing

Monocytes isolated from this protocol were cultured in two clear 96-well plates with RPMI supplemented with 5% Human Sera (Fischer Scientific Catalog #: BP2651700), amphotericin, penicillin, streptomycin (APS). Cells were incubated overnight at 37°C at 5% CO_2 to allow monocyte adherence to the wells.

Monocyte Stimulation with Virus-Like Particles

After overnight incubation, monocytes were treated with the six different VLPs and three control reagents including positive controls; lipopolysaccharide (LPS) and VacV in triplicate; and one section with no VLP or reagent. Plates are incubated overnight at 37°C at 5% CO_2 to allow VLPs to interact with the monocytes. The extent to which monocytes were stimulated is indicated by the amount of interferon secreted into the culturing media. After stimulation, 150 micoliters were harvested and then replaced. This process was repeated every 24 hours for 96 hours. Harvested supernatant was frozen until assayed.

Interferon Production Measurement

20 microliters of supernatants from the monocyte stimulation assays were added to an IFN-β & NF- κβ reporter THP-1 Dual™ Cells (Invivogen, San Diego, CA). THP-1 Dual Cells and incubated for 48 hours. Using the Molecular Devices Spectramax i3x spectrophotometer and analysis with the SoftMax Pro software, we were able to assay for amounts of secreted luciferase (luminescence) proportional to interferon signaling.

Results

The VLPs were created to stimulate human peripheral blood monocytes and measure production of interferon from each VLP preparation (Table 1). We incorporated wild-type (wt) vesicular stomatitis virus attachment protein G as a control, viral protein 40 (VP40) to produce the VLP, and a constitutively active RIG-I to test for interferon induction in VLP preparations. The GPF88A is a mutated glycoprotein with an amino acid change from Phenylalanine to Alanine at the 88th gene position that no longer functions in entry in order to test for the effects of entry on interferon induction (Martinez et. al, 2015). The RIG-I mutant control has an amino acid change from Lysine to Alanine at the 58th amino acid position which prevents binding to viral K63 polyubiquitin chains preventing IRF3 activation by RIG-I CARDs (caspase activation and recruitment domains) and inhibiting IFN-β production (Jiang et. al, 2012).

To normalize for amounts of VLPs, VLP quantification was measured in Relative Fluorescence Units (RFU) using spectrophotometry (Figure 1). Flag VP40 yielded the highest RFU among the others at 3.32 RFU. Flag VP40 was followed by GPF88A (1) at 2.23 RFU and RIG-I-wt/r-VSV (1) at 1.98 RFU.

To test whether VLPs could induce interferon from human monocytes, VP40+GP, caRIG-I-VP40+GP, muRIG-I-VP40+GP VLPs as well as LPS and a Vaccinia virus (VACV-70) were added into monocyte cultures from two blood donors for four days. Supernatant was removed in 24-hour intervals and stored for testing IFN presence using THP-1 Dual reporter cells.

Relative Luminescence indicated the amount of interferon in the supernatant from the monocyte stimulation with VLP preparations. Lipopolysaccharide (LPS) and vaccinia virus (VACV) both served as positive controls. LPS was used as a positive control given it is highly recognized as a PAMP with extracellular PRRs, specifically TLR-4, with an endotoxic effect on human cells (Bertani & Ruiz, 2018). VACV (InvivoGen VACV-70/LyoVec™) serves a positive control due to its ability to profoundly induce IFN-β .

Measuring bioluminescence is the result of THP-1 DUAL reporter cell secretion of luciferase into the media indicating presence and quantity of interferon. Between the two monocyte groups, Human Monocytes 1 and Human Monocytes 2 (HM1 & 2), HM1 consistently produced IFN in all samples provided. THP-DUAL cells alone had produced 66 RFU. RIG-I wt containing VLPs induced the greatest average IFN-production at 1255.67 and 763 RFU from HM1 and HM2, respectively. MRIG-I produced 56.67 and 303.3 RFU, respectively. Positive controls, LPS and VACV, showed 121 and 170.67 RFU then 214.5 and 0 RFU. VSV-g produced 1.33 and 167.3 RFU. Mutant GPs, GPF88A-1 and GPF88A-2, showed 37 and 62 RFU then 0 and 93 RFU. Flag VP40 yielded 63.3 and 83.67 RFU. Finally, THP-1 Dual Cells alone yielded 66 and 0 RFU.

Conclusion

We successfully created VLP preparations to test in interferon stimulation assays. The VLP preparation that created the most VLPs was the VP40 combined with GP consistent with studies by Dr. Ilhem Messaoudi at University of California-Riverside, California, USA (2015) and Dr. Laura Falasca with National Institute for Infectious Diseases, Lazzaro Spallanzani, Rome, Italy (2015). Spectrophotometry values (RFU) when measuring amount of VLPs and IFN are qualitative measurements of quantity and do not enumerate particles or concentration of IFN in solution.

Magnitude of interferon production from each VLP was compared with the positive controls, LPS and VACV. The RIG-I wild type containing VLP with the first human monocyte group yielded 10 times the amount of interferon production in monocyte stimulation when compared to LPS. LPS still didn't yield nearly as much interferon alone as the supplemented RIG-I wt did. THP-1 DUAL Cell blank, or the negative control, in the first human monocyte group exhibited luciferase secretion likely due to minute stimulation from the media or basal luciferase secretion independent of stimulatory material. mRIG-I, being the second highest interferon stimulator within the second human monocyte group, induced 1.7 times the amount of interferon in comparison to LPS. We surmise that newly plated monocytes are less reactive to positive controls due to lack of development in culture, expression of associated PRR's, or activation of other molecular mechanisms required for a substantial reaction. Ultimately, increased interferon production with the VP40/RIG-I wt/GP VLP preparation was attained as a result of adding a constitutionally active RIG-I. The combination of GP, and its capacity to mediate entry into the target monocyte of VLPs that release active RIG-I into the cytoplasm

leads to the production of interferon and potentially to enhance the cellular and humoral immune response. While the wild type RIG-I remained consistent, the mutated GP (GPF88A) showed its inability to properly enter the monocyte to induce production of IFN. Overall, our results are consistent with our hypothesis that the addition of constitutionally active RIG-I induces interferon production from human monocytes, which may enhance vaccine efficacy. Additionally, it provides additional understanding on the effect of viral particles in vaccines with the incorporation of PRRs as supplemental adjuvants for vaccine development.

References

Awate, Sunita, et al. "Mechanisms of Action of Adjuvants." *Frontiers in Immunology*, Frontiers Media S.A., 16 May 2013, www.ncbi.nlm.nih.gov/pmc/articles/PMC3655441/.

Bache, Bache Emmanuel, et al. "Safety, Immunogenicity and Risk–Benefit Analysis of RVSV-ΔG-ZEBOV-GP (V920) Ebola Vaccine in Phase I–III Clinical Trials across Regions." *Future Microbiology*, 7 Feb. 2020, www.futuremedicine.com/doi/10.2217/fmb-2019-0237?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dwww.ncbi.nlm.nih.gov&.

Bertani, Blake, and Natividad Ruiz. "Function and Biogenesis of Lipopolysaccharides." *EcoSal Plus*, U.S. National Library of Medicine, Aug. 2018, www.ncbi.nlm.nih.gov/pmc/articles/PMC6091223/.

Cooper, Christopher L, et al. "T-Cell-Dependent Mechanisms Promote Ebola VLP-Induced Antibody Responses, but Are Dispensable for Vaccine-Mediated Protection." Emerging Microbes & Infections, U.S. National Library of Medicine, 7 June 2017, pubmed.ncbi.nlm.nih.gov/28588288/.

Eisele, Pamela. "First FDA-Approved Vaccine for the Prevention of Ebola Virus Disease, Marking a Critical Milestone in Public Health Preparedness and Response." *U.S. Food and Drug Administration*, FDA, 20 Dec. 2019, www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-ebola-virus-disease-marking-critical-milestone-public-health.

Falasca, L, et al. "Molecular Mechanisms of Ebola Virus Pathogenesis: Focus on Cell Death." *Cell Death and Differentiation*, Nature Publishing Group, Aug. 2015, www.ncbi.nlm.nih.gov/pmc/articles/PMC4495366/.

Faye, Oumar, et al. "Use of Viremia to Evaluate the Baseline Case Fatality Ratio of Ebola Virus Disease and Inform Treatment Studies: A Retrospective Cohort Study." *PLoS Medicine*, Public Library of Science, 1 Dec. 2015, www.ncbi.nlm.nih.gov/pmc/articles/PMC4666644/.

Fuenmayor, J., et al. "Production of Virus-like Particles for Vaccines." *New Biotechnology*, Elsevier, 1 Aug. 2017, www.sciencedirect.com/science/article/abs/pii/S1871678416325511.

Jiang, Xiaomo et al. "Ubiquitin-induced oligomerization of the RNA sensors RIG-I and MDA5 activates antiviral innate immune response." *Immunity* vol. 36,6 (2012): 959-73. doi:10.1016/j.immuni.2012.03.022

Kadanali, Ayten, and Gul Karagoz. "An Overview of Ebola Virus Disease." *Northern Clinics of Istanbul*, Kare Publishing, 24 Apr. 2015, www.ncbi.nlm.nih.gov/pmc/articles/PMC5175058/.

Marcinkiewicz, Janusz, et al. "Ebola Haemorrhagic Fever Virus: Pathogenesis, Immune Responses, Potential Prevention." Folia Medica Cracoviensia, U.S. National Library of Medicine, 2014, www.ncbi.nlm.nih.gov/pubmed/25694094.

Martines, Roosecelis Brasil, et al. "Tissue and Cellular Tropism, Pathology and Pathogenesis of Ebola and Marburg Viruses." *The Journal of Pathology*, U.S. National Library of Medicine, Jan. 2015, www.ncbi.nlm.nih.gov/pubmed/25297522.

Martinez, Osvaldo, et al. "Ebola Virus Exploits a Monocyte Differentiation Program To Promote Its Entry." *Journal of Virology*, American Society for Microbiology Journals, 1 Apr. 2013, jvi.asm.org/content/87/7/3801.

Martinez, Osvaldo, et al. "A Mutation in the Ebola Virus Envelope Glycoprotein Restricts Viral Entry in a Host Species- and Cell-Type-Specific Manner." *Albert Einstein College of Medicine*, American Society for Microbiology, 6 Oct. 2015, einstein.pure.elsevier.com/en/publications/a-mutation-in-the-ebola-virus-envelope-glycoprotein-restricts-vir-2.

Messaoudi, Ilhem, et al. "Filovirus Pathogenesis and Immune Evasion: Insights from Ebola Virus and Marburg Virus." *Nature Reviews Microbiology*, U.S. National Library of Medicine, Nov. 2015, www.ncbi.nlm.nih.gov/pmc/articles/PMC5201123/.

Moller-Tank, Sven, and Wendy Maury. "Ebola Virus Entry: a Curious and Complex Series of Events." *PLoS Pathogens*, Public Library of Science, 30 Apr. 2015, www.ncbi.nlm.nih.gov/pmc/articles/PMC4415789/.

Ploquin, Aurélie, et al. "Ebola Immunity: Gaining a Winning Position in Lightning Chess." *Journal of Immunology (Baltimore, Md. : 1950)*, U.S. National Library of Medicine, 1 Aug. 2018, www.ncbi.nlm.nih.gov/pubmed/30038036.

Regules, Jason A, et al. "A Recombinant Vesicular Stomatitis Virus Ebola Vaccine." *The New England Journal of Medicine*, U.S. National Library of Medicine, 26 Jan. 2017, www.ncbi.nlm.nih.gov/pubmed/25830322.

Salata, Cristiano, et al. "Ebola Virus Entry: From Molecular Characterization to Drug Discovery." Viruses, MDPI, 19 Mar. 2019, www.ncbi.nlm.nih.gov/pmc/articles/PMC6466262/.

Soday, Lior, et al. "Quantitative Temporal Proteomic Analysis of Vaccinia Virus Infection Reveals Regulation of Histone Deacetylases by an Interferon Antagonist." *Cell Reports*, Cell Press, 7 May 2019, www.sciencedirect.com/science/article/pii/S2211124719305078.

Spiropoulou, Christina F, et al. "RIG-I Activation Inhibits Ebolavirus Replication." *Virology*, U.S. National Library of Medicine, 15 Sept. 2009, www.ncbi.nlm.nih.gov/pubmed/19628240.

Warfield, Kelly L., et al. “Ebola Virus-like Particles Protect from Lethal Ebola Virus Infection.” PNAS, National Academy of Sciences, 23 Dec. 2003, www.pnas.org/content/100/26/15889.

Yu, Dong-Shan, et al. “The Lifecycle of the Ebola Virus in Host Cells.” *Oncotarget*, Impact Journals LLC, 15 June 2017, www.ncbi.nlm.nih.gov/pmc/articles/PMC5589696/.

Figures and Tables

Table 1. Plasmid Preparations for Transfection and Monocyte Stimulation

Plasmid Preparation	Volume DNA (uL)	Volume CaCl2 (uL)	Volume Sterile H20 (uL)
P1			
RIGI-Wt	19.31	144	427.1
EBOV GP	4.21		
VP40 B-Lactamase	5.37		
P2			
RIGI M2-1	19.31	144	425.3
EBOV GP	4.21		
VP40 B-Lactamase	5.37		
P3			
RIGI-Wt	19.31	144	415.3
GP F88A	16		
VP40 B-Lactamase	5.37		
P4			
RIGI-Wt	19.31	144	415.3
GP F88A	16		
VP40 B-Lactamase	5.37		
P5			
Flag VP40	22.68	144	423.7
EBOV GP	4.21		
VP40 B-Lactamase	5.37		
P6			
VSV-g	23.81	144	416.1
VP40 B-Lactamase	5.37		

Figure 1. VLP Quantification in Relative Fluorescence Units

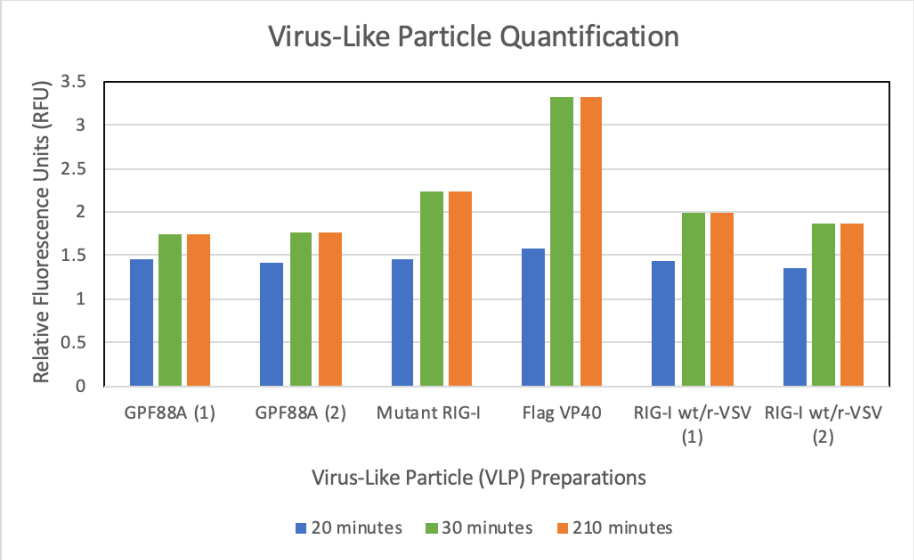

Figure 2. THP-1 Dual Reporter Cells Interferon Measurement in Relative Fluorescence Units