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Human Cytomegalovirus Cytoplasmic Virion Assembly Complex: Structure In Vivo And Role Of Pul103 In Its Biogenesis

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HUMAN CYTOMEGALOVIRUS CYTOPLASMIC VIRION ASSEMBLY COMPLEX: STRUCTURE IN VIVO AND ROLE OF pUL103 IN ITS BIOGENESIS

by

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CHAPTER 1 GENERAL BACKGROUND

Human Cytomegalovirus

Epidemiology. Human cytomegalovirus (HCMV) is one of the many members of the *Herpesviridae*. It is a worldwide opportunistic pathogen typically acquired early in life. It is estimated that 40-100% of the population have HCMV antibody present in their blood. Seroprevalence increases with age and approximately 85% of adults over the age of 70 is HCMV seropositive (1). Other factors associated with increased seroprevalence include ethnicity, socioeconomic status, and living in crowded conditions. Primary infections are asymptomatic in majority of cases. However, immunocompromised patients are at higher risk for serious manifestations as well as recurrence. Illness may manifest as an influenza-like illness to severe disease with multi-system organ failure or dysfunction. The protracted effects alone of HCMV disease on these high-risk populations warrants a development of improve treatment and prevention of this viral infection.

HCMV is transmitted from person to person through a variety of ways which include contact with infected body fluids including saliva, tears, breast milk, urine, and genital secretions. Seroconversion has been documented between family members and individuals in day care centers where unhygienic habits of children make it easy to transmit the virus; prolonged viral shedding is common in children (2). Sexual activity plays a role in acquiring HCMV infection. Since the virus is present in genital secretions, having multiple sexual partners or a history of high-risk sexual activity increases the likelihood of infection. The virus can also be excreted asymptomatically, thus, frequent shedding in saliva and urogenital secretions enable asymptomatic transmission of the virus (2).

HCMV is one of the TORCH microbes: a group of microbes that can cross the placenta (*Toxoplasma gondii*, (Other), rubella, HCMV, herpes simplex virus 1 and 2). Fetuses have a high risk of acquiring HCMV disease in utero during primary maternal infection. Also, it can be acquired perinatally from exposure to birth canal secretions during delivery, or postnatally from maternal milk during breast feeding.

In addition, HCMV can also be transmitted via blood products and tissue and organ transplants.

Like all members of the *Herpesviridae*, HCMV exhibits latency that can reactivate periodically especially when the host becomes immunosuppressed. HCMV establishes latency in the progenitors of the myeloid lineage, mainly the monocyte precursors (3). HCMV replication occurs widely in multiple tissues, including neutrophils, leukocytes, and monocytes, which can serve as vehicles for spread (4). Due to its broad tissue tropism, it can cause a wide spectrum of disease.

Clinical disease. Most adults possess HCMV antibodies and are normally asymptomatic. A self-limiting febrile illness is a common presentation during primary infection. Symptomatic infection may present as mononucleosis (also known as heterophile antibody negative mononucleosis to distinguish from that caused by Epstein-Barr virus.) Mononucleosis characterized by fever, chills, sweats, headache and pharyngitis is more commonly observed in young adults. Skin rashes such as macular, papular, or rubelliform spots may accompany these signs and symptoms (3). Common lab anomalies will be atypical lymphocytes, thrombocytopenia, elevated rheumatoid factor and anemia. In some cases, elevated atypical

lymphocytes and liver enzymes could persist for many months. A post-viral fatigue syndrome can occur in some adults (5).

For the immunocompromised host, the primary HCMV infection replicates uncontrollably and can lead to disseminated infections. HCMV infection in immunocompromised hosts can have different clinical manifestations depending on the population. Seropositive bone marrow transplant patients, or those receiving a positive donor bone marrow, have a high risk of developing severe HCMV pneumonitis that can lead to death. The presentation of pneumonitis is presumed to be a pathological consequence from the host immune response against the virus (3). AIDS patients commonly develop viremia, progressive retinitis that may lead to blindness, and diarrhea from HCMV colitis. Further, HCMV can disseminate to the brain causing encephalitis (3).

HCMV colitis is uncommon in immunocompetent people. However in the immunocompromised host, it can lead to fulminating colitis manifesting as severe bloody diarrhea, abdominal pain, and fevers. Diagnosis requires a high degree of clinical suspicion and a biopsy is generally needed in almost all cases (6).

Other clinical syndromes described with HCMV is pericarditis and myocarditis. Post HCMV Guillain-Barré syndrome is a well described but uncommon sequela following resolution of infection (5).

Congenital HCMV. HCMV is the most common cause of congenital infections, about 1% of live births, and approximately 10 percent of those are symptomatic at birth (7). If due to primary maternal infection, the fetus is more likely to be symptomatic at birth or experience long-term sequelae which can include progressive hearing loss, visual and intellectual

impairment, severe disease including organ damage, CNS abnormalities, or death. Disease manifestation is most severe if primary infection was acquired during the first trimester of fetal development. Sensorineural hearing loss (SNHL) is the most common sequelae of congenital disease caused by viral spread into the inner ear structures forming a focus of infection accompanied by inflammation. Other signs and symptoms include thrombocytopenia purpura, hepatitis, pneumonitis and myocarditis (3).

HCMV infection in the elderly. In immunocompetent adults, illness is generally self limiting. It is one of the viral illness which can present as fever of unknown origin lasting for more than 4-6 weeks with absence of lab anomalies. In such cases, diagnosis is difficult and other infections, malignancies and cancers have to be ruled out (8).

Lab Diagnosis. HCMV can be detected in a variety of specimens: tissue, urine, respiratory secretions, CSF, peripheral blood, and other body fluids. However, since most of us can shed this virus asymptomatically, diagnosis of symptomatic infection is best confirmed by detection of HCMV DNA from sterile body fluids (*e.g.*, peripheral blood and its components, CSF.) PCR method is the gold standard for testing owing to its high sensitivity and faster turn around time compared to cell cultures. False positive can occur and interpretation of PCR results should correlate with clinical symptoms, and in some cases, an H&E tissue biopsy confirmation may be necessary (9).

Other methods available include detection of IgG or IgM antibodies by serology (*e.g.*, ELISA), detection of HCMV pp65 antigen in leukocytes, growth in human fibroblast cell cultures, and histopathology. In histopathology slides, HCMV exhibits a characteristic “Owl’s eye” intranuclear inclusions in a cytomegalic infected cell (Fig. 1), although this feature may be

absent in some infected organs. Biopsies may be performed if organ involvement is suspected, or in cases of disseminated infections.

Diagnosis of congenital HCMV can be done in utero by amniocentesis if the infection is suspected due to primary maternal infection or from abnormal findings on the ultrasound. The sensitivity of this sample depends on the timing of collection: there is higher sensitivity if collected after 21 weeks of gestation and a 6 weeks lag time between maternal infection and collection that takes into account replication of the virus in the placenta, transmission to the fetus and consequent replication in the fetal kidney and excretion into the amniotic fluid (10). The amniotic fluid is then tested for the presence of HCMV DNA using PCR.

For perinatal diagnosis, the standard method is a PCR test on saliva collected within 3 weeks of birth. This is followed by a urine test for confirmation. The saliva must be collected at least 1 hour after breast feeding because HCMV-positive mothers can shed HCMV in their breast milk and may cause a false positive result on the saliva sample collected soon after breast feeding (10).

Molecular. HCMV is a betaherpesvirus with an enveloped virion that houses a linear double-stranded DNA. At approximately 230 kbp, it is the largest genome among the human herpesviruses. Primary attachment for entry requires the viral glycoproteins gB, gH, and gL that attach to the heparan sulfate proteoglycan receptors on the host cells. The virus may either enter a lytic cycle, or go latent. The lytic replication cycle has three phases beginning with the expression of the immediate early genes that code for regulatory proteins, early genes that code for proteins used for replication, and the late genes that regulate the structural proteins to make complete virions. The permissiveness of a cell for the lytic cycle or the pathogenesis of an

HCMV infection involves complex interactions between the virus and its host. The large HCMV genome encodes genes that allow the virus to counteract many host defenses.

Treatment and Prevention. Cell-mediated immunity is the primary immune response required to control HCMV infection, which explains why the high-risk groups for severe disease are those who have deficiencies in CD4+ and CD8+ lymphocytes. Common antiviral drugs used against HCMV (*i.e*., foscarnet, valganciclovir, ganciclovir) are highly toxic and are only used to treat high risk groups such as those with congenital disease and infections in immunocompromised hosts. No vaccines are currently available. Preventative measures to curtail the risk of transmission include good hand washing/proper hygienic practices, and using seronegative donor blood or tissue products (11). A deeper understanding of the effects of HCMV and the mechanism of its pathogenicity on infected cells is vital to the development of vaccines and new antiviral drugs that are less toxic.

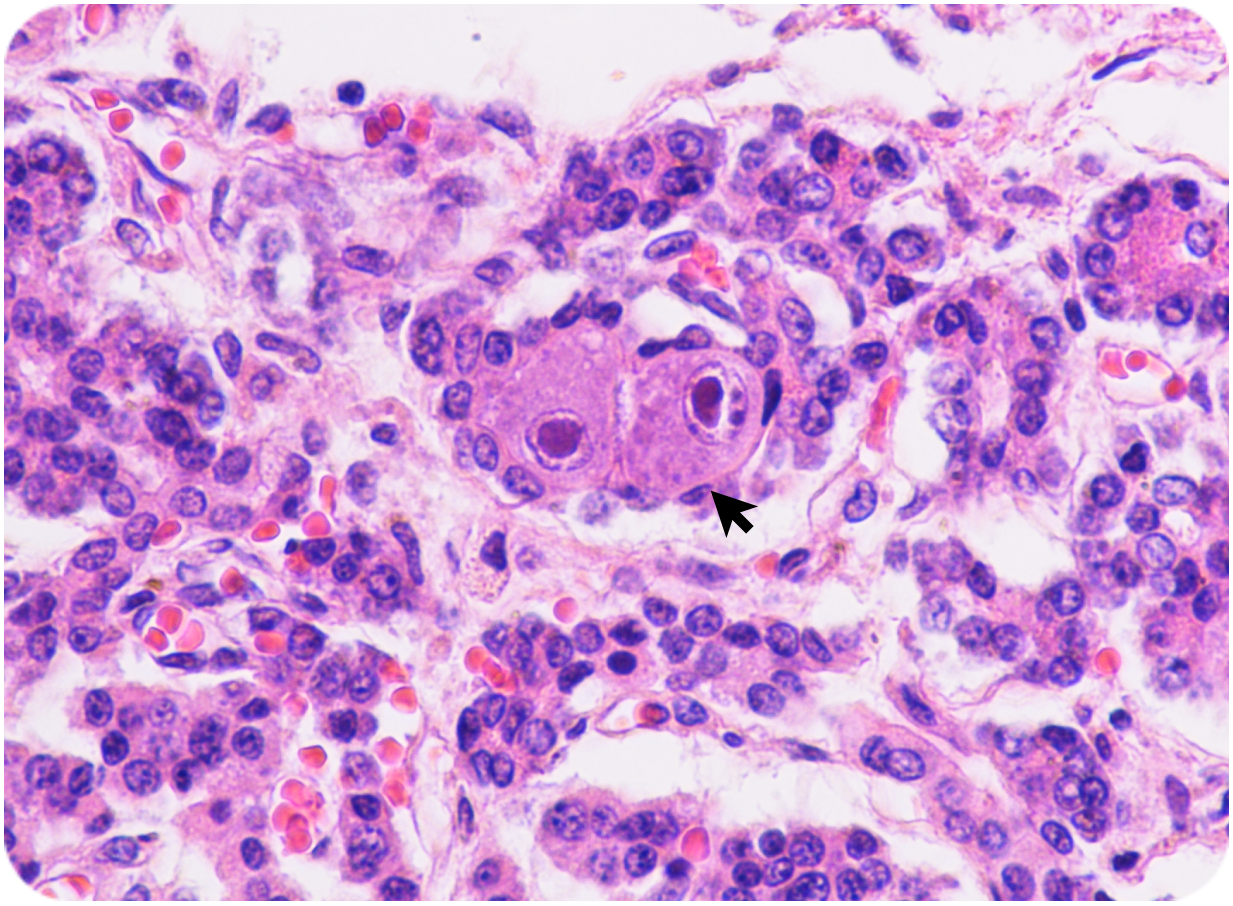


FIG 1 Pancreas H&E stain showing Owl's eye morphology (arrow) of an infected cell. 40X cropped. Tissue slide from Detroit Children's Hospital.

CHAPTER 2 DETECTING THE cVAC STRUCTURE IN VIVO

Introduction

HCMV induces cytopathic effects in host cells typically characterized by an enlarged kidney-bean shaped nucleus bent around a Golgi ring with the endosomes at the center forming the structure known as the cytoplasmic virion assembly complex (cVAC) (Fig. 2). The cVAC is where virus particles are assembled and trafficked out of the cell. This structure has been elucidated and demonstrated in vitro in multiple studies (12-14), however, no in vivo studies have been done. Cellular markers that detect the cVAC structure include the Golgi, trans-Golgi, and early endosomes. We aim to detect by immunofluorescence assay some of the cellular and viral components that form the cVAC to learn if the formation of this complex can be observed in vivo.

Materials and Methods

Materials. 12 sets of formalin-fixed, paraffin-embedded tissue slides from children with congenital HCMV were obtained from Detroit Children's Hospital courtesy of Dr. Janet Poulik, M.D., Department of Pathology, Detroit Medical Center, with Institutional Review Board (IRB) approval. Dr. Noman Hussain (Fellow in Infectious Diseases) was instrumental in getting the approval from the IRB and helped during the process of developing the experimental methods.

The tissue slides were taken from various infected anatomical sites: lung, colon, stomach, intestines, trachea, etc., and the quantity of infected cells in each set were assessed using a Hematoxylin & Eosin (H&E) stained slide. Slides that exhibit more than 2 infected cells per 40X field were used for the experiment. In addition to the slides from congenitally infected children, commercially prepared placenta (Azer Scientific) and lung (StatLab) tissue slides were

also used in the early phase of the experiment. Commercially prepared CMV infected control slides (Bion, Inc) were used to verify the functionality of the antibodies in each experiment (Fig. 3)

Deparaffinization, rehydration, and antigen retrieval. Based on preliminary experiments, the optimized protocol described in this section was used.

Paraffin used to solidify the tissue sections for slicing must be removed first prior to staining to allow buffers and antibodies to penetrate. This was done by baking the slides at 50°C for 1 hour to melt the paraffin wax followed by three xylene washes at 5 minutes each. Next, xylene was removed with two 100% ethanol washes, then the tissue slides were hydrated gradually through 95% and 70% alcohol washes and two washes with distilled water. The formalin fixative used to preserve the integrity of the tissue sections induces the formation of methylene bridges between proteins that may potentially block antigenic sites. Heating the slides can cleave these cross-links between proteins (15). This was accomplished with a heat-induced antigen retrieval (HIER) method by immersing the slides in a buffer jar inside an 80-90 °C water bath. As the heating and subsequent cooling process cause folding and re-folding of the polypeptides, masked epitopes are exposed. The re-folding of the polypeptide chains can be affected by the buffer pH which determines how the positive- and negatively-charged polypeptides react with each other (15). We made our own buffers using Abcam's recommendations (16). In addition, we used R-Universal Epitope Recovery Buffer (Electron Microscopy Sciences). As shown in Tables 1 and 2, we tried different duration and temperature of baking and antigen retrieval, and explored different buffers to optimize the deparaffinization, rehydration, and antigen-retrieval steps for our tissue slides.

Immunofluorescent staining. After the HIER step, the slides were washed with PBS and autofluorescence was quenched by incubation in 50 mM ammonium chloride for 30 minutes. This was followed by permeabilization in blocking serum with 0.2% Triton-X for 25 minutes, incubation in blocking serum only for 1 hour, then stained with primary antibodies overnight at 4°C (antibodies described in Table 3). The slides were then stained with fluorescent-tagged secondary antibodies for 1 hour at room temperature (Alexa Fluor 488-conjugated goat anti-mouse IgG and Alexa Fluor 568-conjugated goat anti-rabbit IgG, both from Molecular Probes, Carlsbad, CA). The slides were mounted with Vectashield containing DAPI (4', 6-diamidino-2-phenylindole) (Vector Laboratories Inc., CA). Imaging was done on a Nikon E800 fluorescence microscope. As shown in Table 2, variations in the duration of incubation were performed to specifically optimize the staining procedure for our slides.

Results

The ability to visualize cellular structures varied with the quality of the slides and the staining process performed. Initial efforts included identification of the optimal procedure to clear the paraffin from the different types of tissue slides, detecting the epitopes of interest, and quenching the autofluorescence typically observed in tissue sections. Once we developed the optimum experimental procedure by trying different combinations of the factors shown in Tables 1 and 2, (optimal protocol is as described in the Deparaffinization…, and Immunofluorescent staining sections above), we were able to detect good nuclear IE2 staining in most of the slides (Figs. 4 and 5). Other viral proteins tested such as glycoprotein B (gB) and pp28 were also detected (Figs. 5A, 5B) which proved the ability of the procedure to detect HCMV infected cells. As expected, the tissue sections with more infected cells per field (*i.e.*, colon, ovary, pancreas)

provided better results. Fig. 6 shows a classic kidney-bean shaped infected colon cell with staining of the Golgi and early endosomes evident in the area of the cVAC.

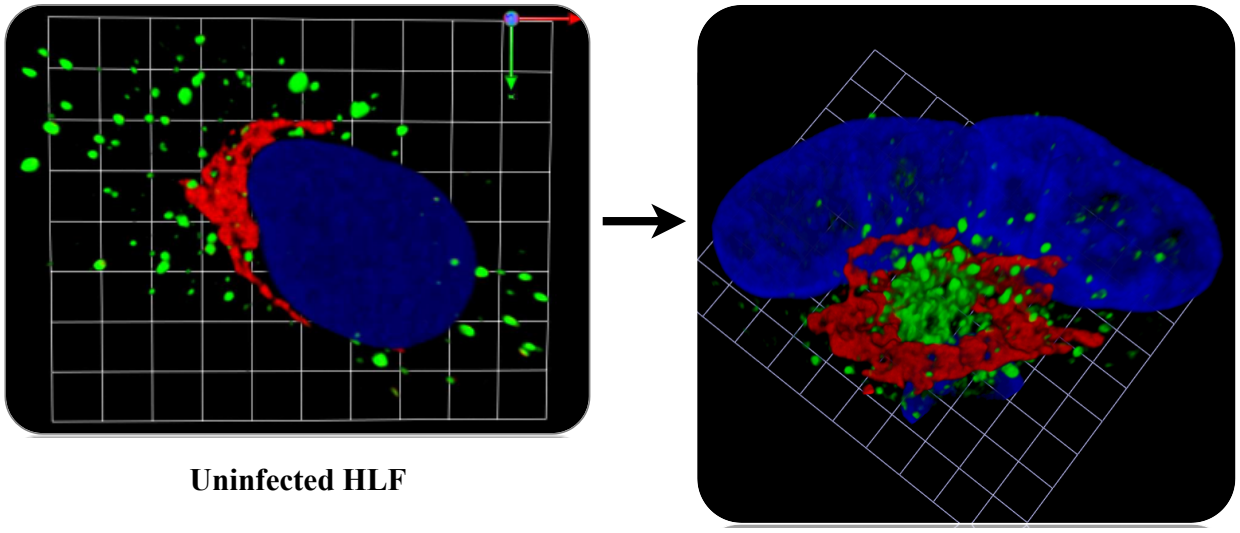
Staining of the Golgi and early endosomes, structures classically associated with the cVAC, were not consistently evident in every slide tested. This could be attributed to the three-dimensional nature of the tissue sections which affects the orientation of the infected cell seen microscopically; an example is shown on Fig. 7 (12).

Discussion

The objective of this work was to detect the cVAC structure in FFPE tissue slides. By developing an optimized staining procedure, we discovered that the cVAC is formed in tissue specimens.

An important aspect to keep in mind when working with tissue slides as opposed to a monolayer of cells grown in culture is the three dimensional aspect of the specimen. As the FFPE section is sliced, the structures of interest could be severed and exhibit a shape or form that is not consistent with their typical morphology. Also, the angle of the cell on the tissue could hide expected features. Fig. 6 from our experiment suggests that the cVAC is indeed present in tissue as it is in cell cultures. More robust evidence is needed however, and would require a more extensive and three-dimensional microscopic study of ideally-positioned infected cells in tissue sections. A limitation of this study was the amount of acceptable slides available for testing. A majority of the slides contained rare to very few scattered infected cells and were not ideal for testing. Interesting to note, this “scattered infection” seems to be a feature consistent with how HCMV establishes an infection. Mayer et al. recently reported that HCMV spreads to neighboring cells inefficiently and normally requires numerous tries to establish an infection

(17). This phenomenon can be observed on Fig. 1 which shows 2 infected cells in the center surrounded by many uninfected cells.



Uninfected HLF

HCMV in HLF cells 5 dpi

DAPI (Nucleus)
GM130 (Golgi)
EEA1 (Early/recycling endosomes)

FIG 2 HCMV induces cytopathic effects in host cells forming the cVAC. (Left) Uninfected HLF cell (human lung fibroblast) with the Golgi adjacent to the nuclei and the early endosomes scattered around the cell. (Right) Infected cell 5 dpi (days post infection) showing an enlarged bent nucleus with the Golgi forming a ring structure and the endosomes localized in the center. Das and Pellett, J. Virol. 2011.

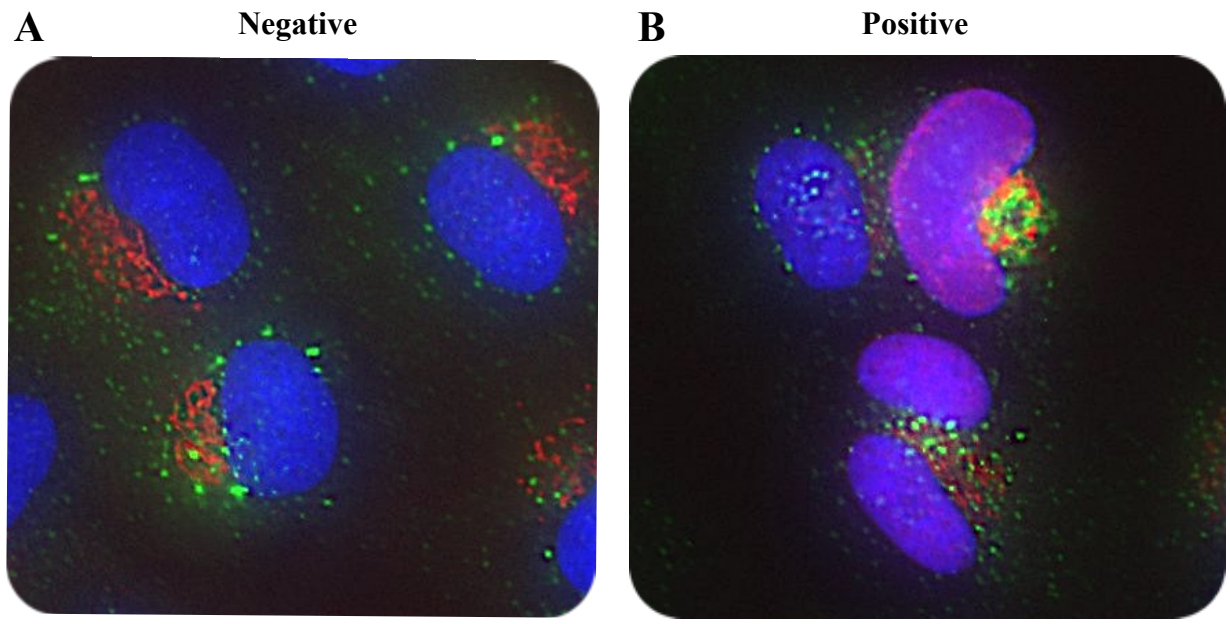


FIG 3 Commercial fibroblast control slides that show staining of the corresponding antibodies. (A) Negative control with cells staining for the cellular markers GM130 and EEA1. (B) Positive control showing the classic large kidney-bean shaped nucleus on top right staining with the viral nuclear marker IE2, and the cellular Golgi and EEA1 markers localized in the center, as expected.

TABLE 1 List of buffers used

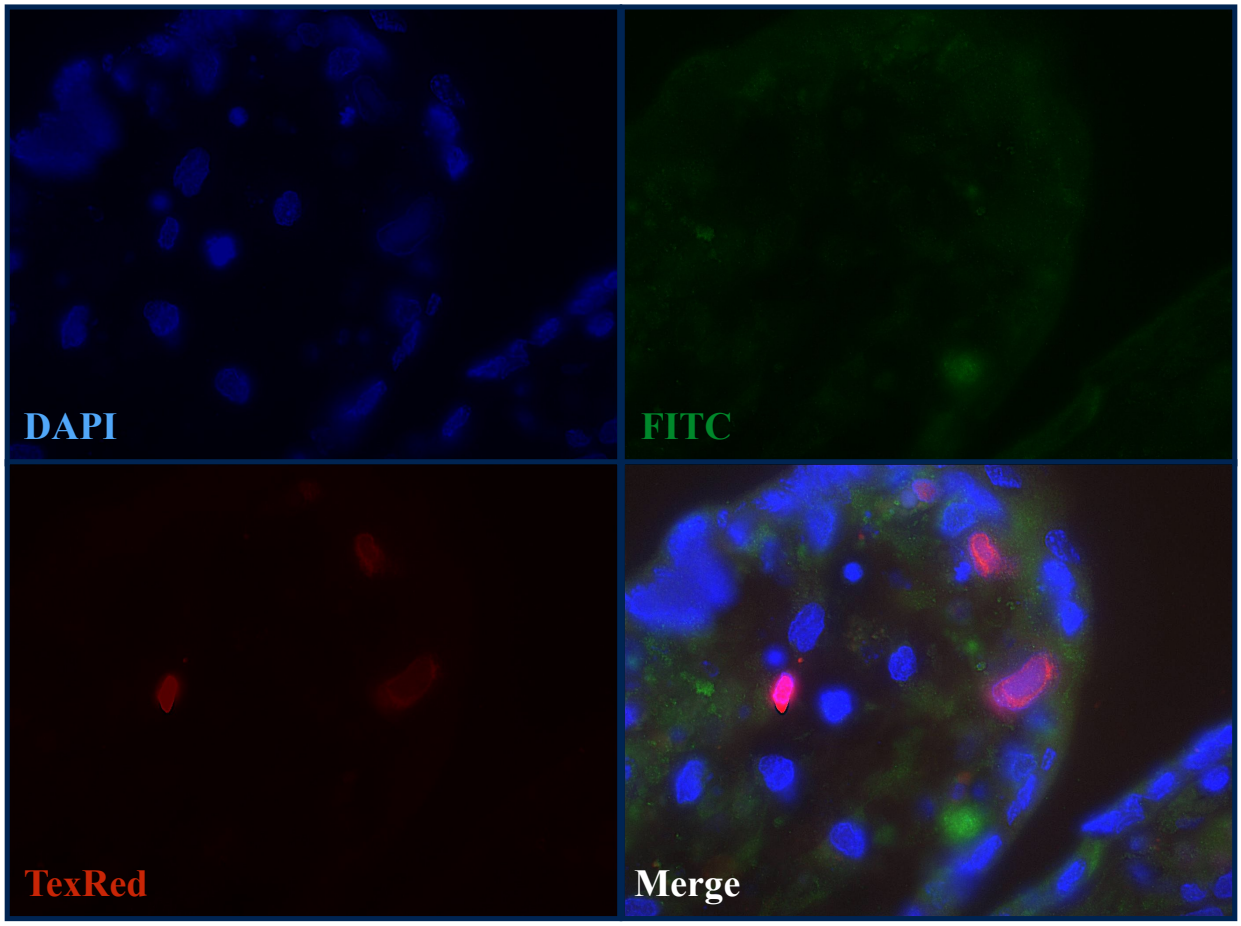
Sodium citrate pH 6.0	**EDTA pH 8.0**	**Tris base pH 9.0**	**R-Universal**
10 mM sodium citrate, 0.05% Tween 20, pH 6.0 Abcam recipe: 2.94 g Tri-sodium citrate (dihydrate) dissolved in 1 L of deionized water. pH adjusted to 6.0 with 1N HCl. 0.5 ml Tween 20 added.	1 mM EDTA, pH 8.0 Abcam recipe: 0.37 g EDTA dissolved in 1 L of deionized water. pH adjusted to 8.0 with NaOH	10 mM Tris base, 1 mM EDTA solution, 0.05% Tween 20, pH 9.0 Abcam recipe: 1.21 g of Tris and 0.37 g of EDTA dissolved in 1 L of deionized water. pH adjusted to 9.0 with NaOH. 0.5 ml Tween 20 added.	R-Universal Epitope Recovery Buffer (Electron Microscopy Sciences)

TABLE 2 Experimental conditions

Step	**Variations**
Bake slides	not performed 50-55 °C for 1 hour
Heat-induced antigen retrieval incubation period	0 minutes 20 minutes 30 minutes 1 hour
Heat-induced antigen retrieval water bath temperature	60 °C 85-90 °C
Triton-X permeabilization step incubation period	0 minutes 15 minutes 25 minutes
Blocking step duration with 50 mM ammonium chloride	0 minutes 15 minutes 30 minutes
Primary antibody incubation period	1 hour in room temperature; antibody diluted in blocking buffer only 1 hour in room temperature; antibody diluted in blocking buffer with Triton-X Overnight incubation at 4 °C; antibody diluted in blocking buffer only
Secondary antibody incubation period	1 hour in room temperature

TABLE 3 Primary antibodies used in immunofluorescence assay

Antibody Target	Host/Isotype, clone	Source, catalog no.
Epitope tag		
V5 (14 amino acids)	Mouse monoclonal/IgG2aκ	LifeTechnologies, R960-25
Cellular		
GM130 (130kDa)	Mouse monoclonal/IgG1(κ), clone 35/GM130	BD Biosciences, 610822
EEA1 (180kDa)	Rabbit polyclonal	Abcam, ab2900
Mann II	Rabbit polyclonal	Abcam, ab12277
HCMV		
IE2 (86kDa)	Mouse monoclonal, clone 8B1.2	Chemicon (Millipore), **MAB810**
pUL99 (pp28)	Mouse monoclonal/IgG2A(k), 5C3	Virusys, CA004-100
gB (55 kDa and 110 kDa)	Mouse monoclonal/ IgG1(k), clone 2F12	Virusys, CA005-100



DAPI (Nucleus)
IE2 (Nucleus), GM130 (Golgi)
EEA1 (Early/recycling endosomes)

FIG 4 Placenta tissue infected with HCMV. IE2 staining is evident in the infected cells, however, the Golgi and early endosomes are not detected in this slide. Infected cells may not exhibit the typical kidney-bean shaped nucleus but are cytomegalic.

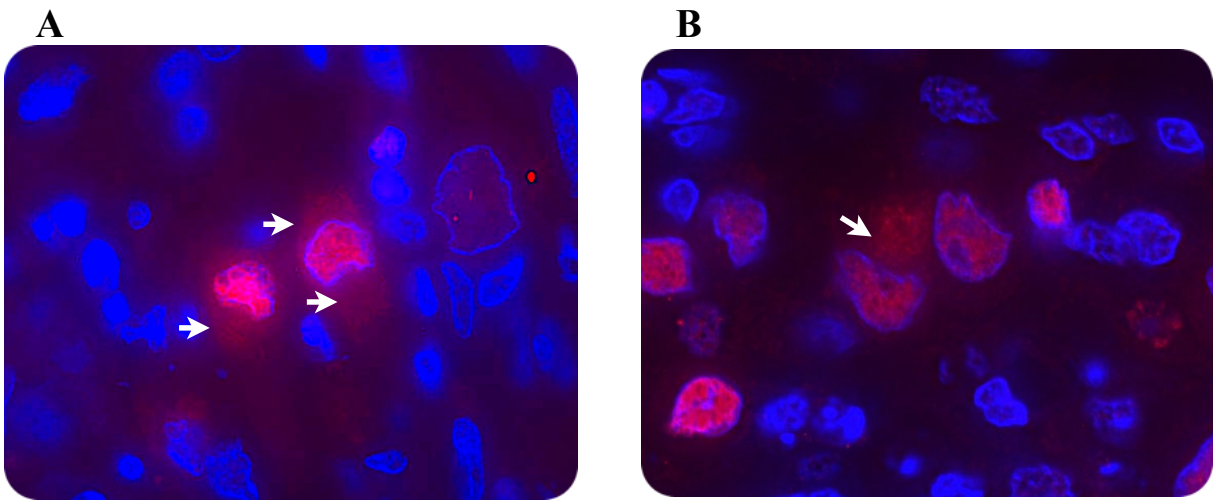
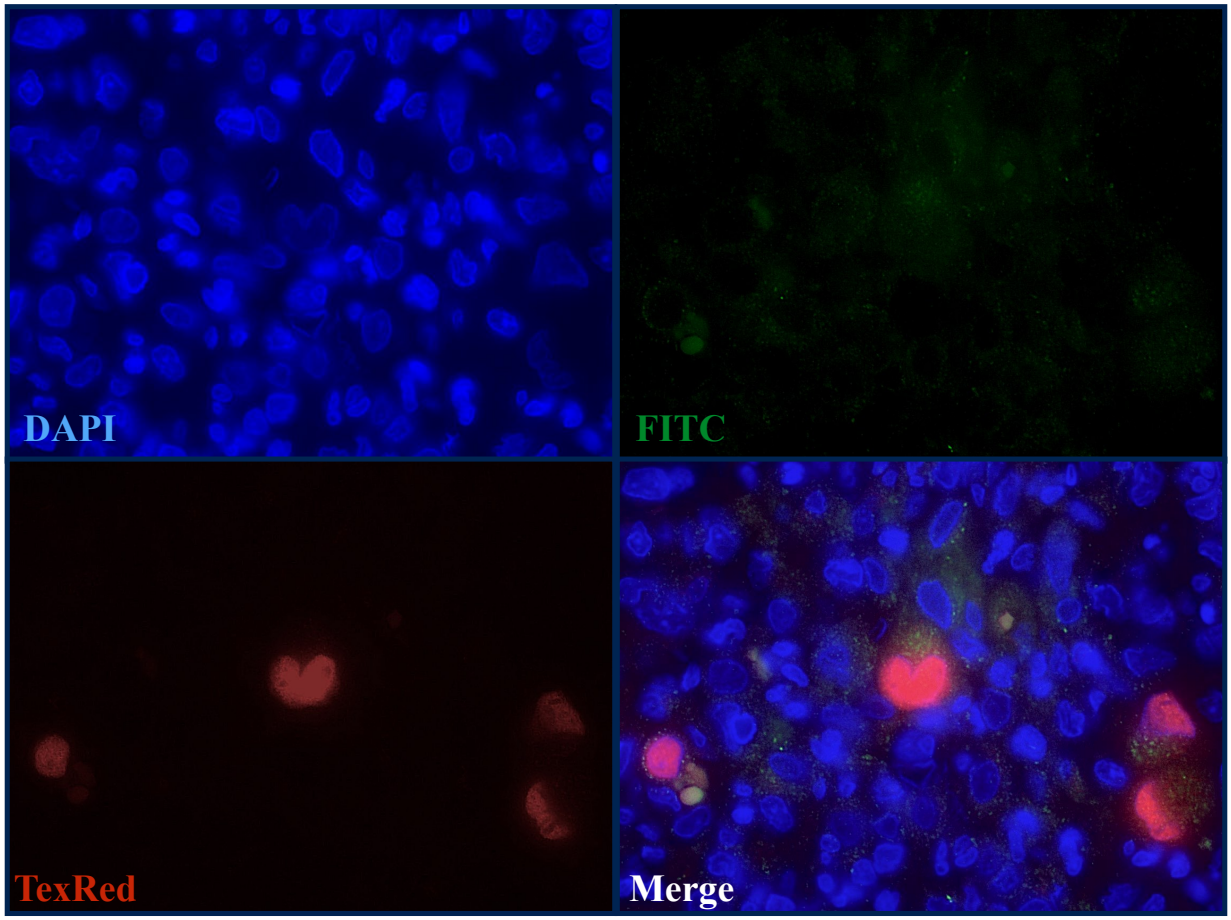


FIG 5 Tissue slides that show positive viral nuclear and cytoplasmic staining. (A) Ovary tissue. Arrows pointing at gB cytoplasmic staining of an infected cell. (B) Placenta tissue. Arrow pointing at pp28 cytoplasmic staining of an infected cell.



DAPI (Nucleus)
IE2 (Nucleus), GM130 (Golgi)
EEA1 (Early/recycling endosomes)

FIG 6 Colon tissue infected with HCMV. Orientation of the tissue section showed the characteristic large kidney-bean shaped nucleus along with staining of the Golgi and early endosomes in the cVAC area.

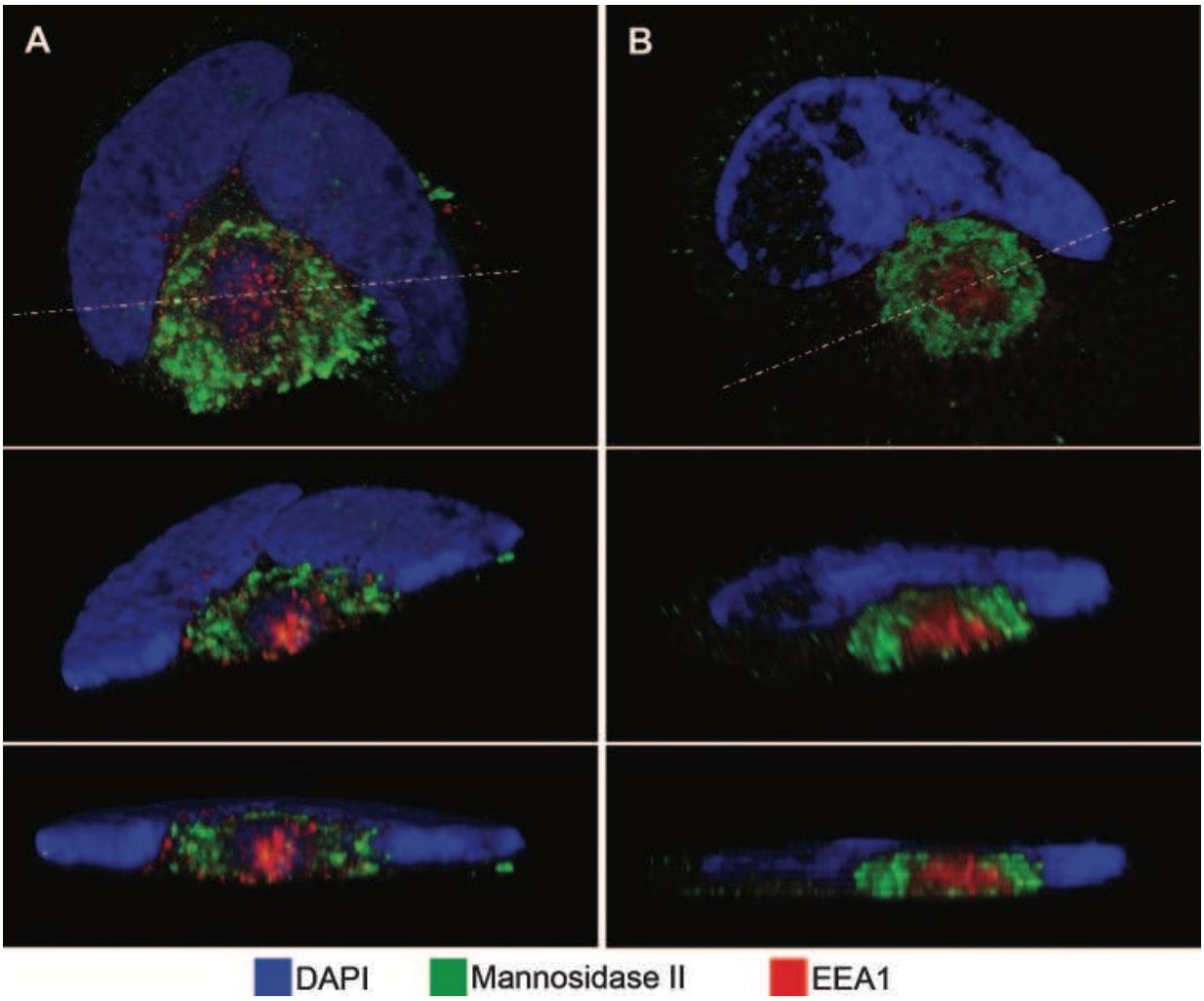


FIG 7 Three dimensional aspects of an HCMV infected cell. Image from Das et al 2007.

CHAPTER 3 ROLE OF pUL103 IN cVAC BIOGENESIS

Introduction

pUL103 is a tegument protein with known functions during the late replication cycle of HCMV. Previous works in the Pellett laboratory have shown that pUL103 is involved in the cVAC biogenesis, cell-to-cell spread, and virion maturation and egress (18). Contributors to the assembly of the cVAC can be potential targets for new antivirals and warrant further study.

Here we try to address the question of whether the presence of pUL103 at early times after infection is critical for cVAC biogenesis. To study the effects of pUL103, pUL103 was fused with a destabilization domain (FKBP) that can be regulated by the presence or absence of Shield-1 ligand. Shield-1 ligand attachment to the pUL103 construct stabilizes and protects it from proteasomal degradation (Fig. 8) (19). The experimental design shown in Fig. 9 anticipates that cVAC biogenesis occurs around 3 days post infection. pUL103 is a late protein and is synthesized just prior to the cVAC, around 48 hours p.i., continuing throughout the course of the infection. Previous siRNA knockdown experiments done in the Pellett lab have shown that less pUL103 is synthesized late in the infection (18) represented in Fig. 9 (pUL103 siRNA) with a thin bar compared to the wild type (pUL103 wt). With our pUL103-FKBP construct (pUL103-FS+), if Shield-1 is present, input pUL103 from infecting virions and newly made pUL103 should be comparable to wild type levels. However in the absence of Shield-1, input pUL103 is degraded more quickly, in addition, less are being synthesized. The experimental design aims to determine whether the input pUL103 plays a role in the re-arranging of the cVAC area of an infected cell and if reducing this amount will have an effect on that process.

Materials and Methods

Human foreskin fibroblast (HFF) cells were seeded at 80% confluency on 0.2% gelatin-coated 8-well glass chamber slides (Thermo Fisher Scientific, Waltham, MA). The next day, the cells were infected with UL103-FKBP-V5 HCMV construct at 0.1 MOI and Shield-1 was added in certain chamber wells under set time points of 0 hpi and then at 12 hour increments as illustrated in Fig. 9. Shield-1 was replenished every 2 days in the corresponding wells. At 144 hpi, the slides were fixed with paraformaldehyde and stained and imaged as previously described in Chapter 2. The number of infected cells with fully formed cVAC were quantified relative to those without.

Results

Table 4 shows the results of the 2 experiments. In Experiment 1, 15% of infected cells have well-formed cVAC in the absence of Shield-1 compared to 37% when Shield-1 was present throughout the course of the experiment. When Shield-1 was omitted until later time points, there was no defect in cVAC formation.

Experiment 2 differed from Experiment 1 in that it had more extended period prior to addition of Shield-1. Experiment 2 has similar observations except that in the No Shield-1 added control the fraction of cells with well-formed cVAC was higher than in Experiment 1 (30% vs. 15%), however, it was still lower than the other time points in the experiment.

Discussion

The objective of these experiments is to elucidate the role of input pUL103 in cVAC biogenesis. Our results indicate that it is not critical for pUL103 to be present at wild type levels during the early time of infection for cVAC biogenesis to occur.

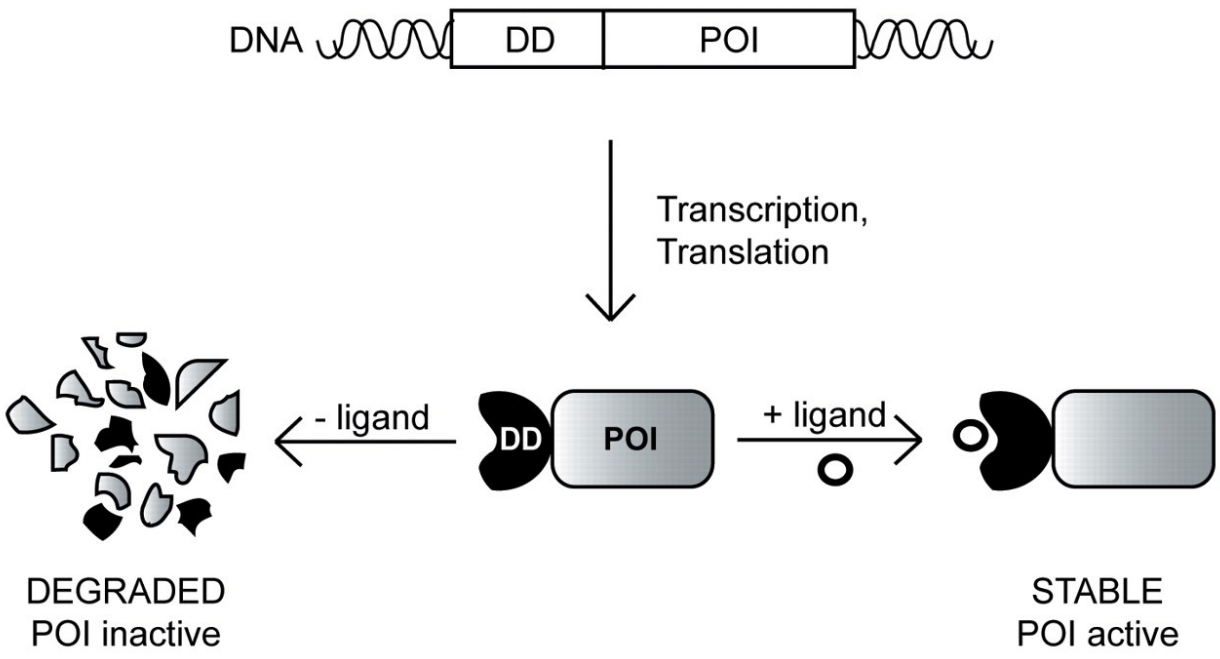


FIG 8 Regulatable Shield-1 ligand protects a protein of interest from proteasomal degradation. Hagan EL, et al. 2009.

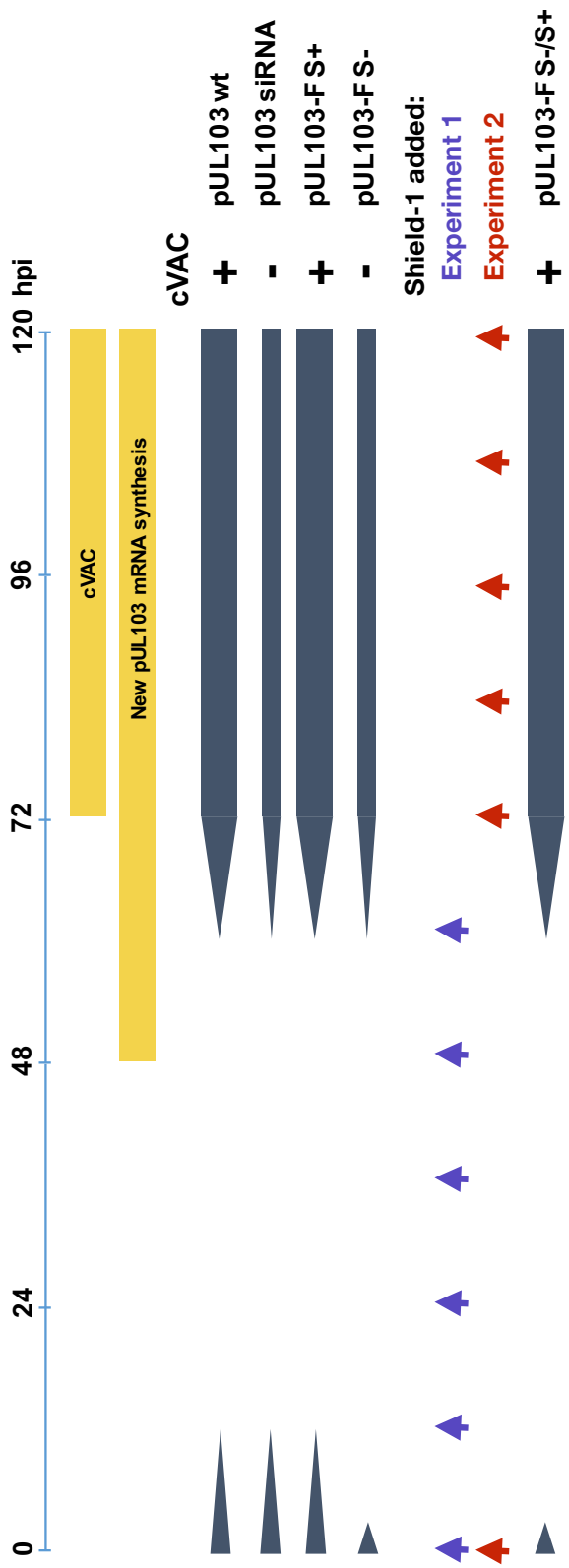
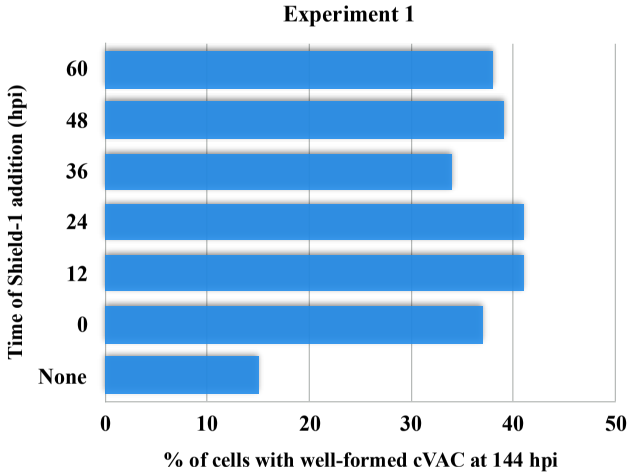


FIG 9 Experimental design to study the effect of pUL103 on the formation of cVAC using a regulatable Shield-1 ligand added at different time points. After addition, Shield-1 was replenished every 2 days. Slides were stained at 144 hpi.

TABLE 4 Effect of pUL103 on cVAC biogenesis in the presence or absence of Shield-1 ligand.

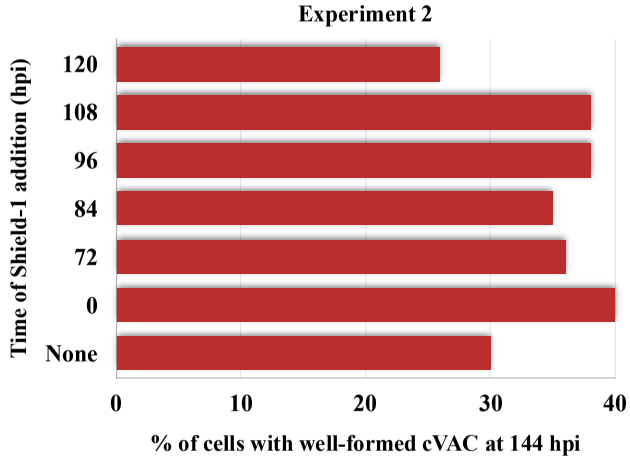
Experiment 1

Time of Shield-1 addition (hpi)	None	0	12	24	36	48	60
IE2 (+) cells counted	45	41	49	69	88	62	52
cells with well-formed cVAC	7	15	16	28	30	24	20
% of cells counted with well-formed cVAC	**15**	**37**	**41**	**41**	**34**	**39**	**38**



Experiment 2

Time of Shield-1 addition (hpi)	None	0	72	84	96	108	120
IE2 (+) cells counted	66	70	66	84	65	77	82
cells with well-formed cVAC	20	28	24	29	25	29	21
% of cells counted with well-formed cVAC	**30**	**40**	**36**	**35**	**38**	**38**	**26**



CHAPTER 4 CONCLUSIONS

Human cytomegalovirus is a prevalent opportunistic pathogen that targets and cause severe disease to those who have a weakened immune system. A hallmark characteristic of its effect on the host cell is enlargement of nuclei and subsequent remodeling of the internal structures to form the cytoplasmic virion assembly complex (cVAC) in the center. The cVAC is the final site of virion maturation and egress, and viral proteins involved in its biogenesis are potential targets for antivirals. To further learn about the pathogenesis of HCMV infection we studied the structure of the cVAC in tissue slides from patients with congenital HCMV and discovered that cVAC forms in tissue similar to cell cultures. Previous work in the Pellett laboratory had identified viral genes important in cVAC biogenesis, one of which is pUL103. pUL103 is a multi-functional tegument protein that plays many other roles in the pathogenesis of the infection. To study its potential role during the early stages of cVAC biogenesis, we conducted time point studies using a pUL103-FKBP construct whose presence in the infection can be regulated with the addition of a Shield-1 ligand. Our findings suggest that input pUL103 at early stages of infection is not critical in the formation of cVAC at later times.

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ABSTRACT

HUMAN CYTOMEGALOVIRUS CYTOPLASMIC VIRION ASSEMBLY COMPLEX: STRUCTURE IN VIVO AND ROLE OF pUL103 IN ITS BIOGENESIS

by

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Human Cytomegalovirus (HCMV) is a linear, double stranded DNA virus that causes severe disease in the immunocompromised, and is one of the common cause of congenital disease. Antivirals that are currently available for treatment target the DNA replication of the virus cycle, and are highly toxic. Finding new drug targets, such as proteins responsible for virion assembly and egress, would help to alleviate the disease burden.

HCMV remodels the host cell to form a structure called the cytoplasmic virion assembly complex (cVAC), a site of virion maturation and egress. The first objective of this work is to study the structure of the cVAC in vivo by immunofluorescence assay using slides from children with congenital HCMV, and we discovered that the cVAC is also formed in vivo. The second objective is to elucidate the role of pUL103 in cVAC biogenesis in the early stages of infection using a regulatable pUL103-FKBP construct and Shield-1 ligand. We found that decreased levels of pUL103 at the early stages of infection is not critical for cVAC biogenesis at later times of the infection.

AUTOBIOGRAPHICAL STATEMENT

A backpacker at heart but always kept a stable job because I was told it is prudent to have roots along with my wings. Like any places I have traveled to, graduate school was a journey that was equally challenging and fun, and is chock-full of learning experiences. I am convinced that I am coming out of this venture a better person than when I came in.

When not traveling, I spend my time working with microbial cultures and teaching young minds the wonderful world of microbiology. Maybe I'll make a difference in some of them, just like my mentors have with me.