Midwestern Journal
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An Immune Evasion Reading List
pg. 13

Opposition

Undergraduate Research
pg. 16

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To The Reader,

I am pleased and honored to present the sophomore edition of the *Midwestern Journal of Undergraduate Sciences*. Our mission remains to provide a rare opportunity for undergraduate students to publish their research and commentary in cases where their work would not otherwise be published at all.

We accept submissions from undergraduate scientists and their mentors seeking to publish their 'free-standing' research projects. Although the MJUS is not currently peer-reviewed in a formal manner, we do review submissions in collaboration with mentor authors to ensure a high level of writing and a scientifically rigorous approach. As the journal matures, we look forward to putting a more independent review process in place.

In this issue, The Educator's Corner consists of a short article on using primary journal articles in undergraduate science education, along with the reading list of articles used in the KU Biotechnology program's Selected Topics course Spring 2023 semester. In the future, the Educator's Corner will be transformed into the reading list for this course and a short introduction to the topic. This course's readings are the source of each issue's theme and review articles by students.

We reiterate our hope that readers, whether they are students or faculty, consider the MJUS for the publication of undergraduate research projects in the natural sciences. Please see our online author's guide for more information on the scope and length of articles and the style of submissions.

-J.F. Treml

Contributors

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Table of Contents

Call for Papers

The Midwestern Journal of Undergraduate Sciences features research designed and performed by undergraduate scientists. The journal addresses a variety of disciplines and focuses on work done as senior capstone projects. We especially encourage works authored or co-authored by community college students and also by students pursuing master's degrees to submit. All submissions are due by the last Friday in May of each year.
For information for authors, as well as our submission site, visit: https://journals.ku.edu/MJUSc/about/submissions.

A Semester of Immunoregulation in Review

By J.F. Treml

The Biotechnology program at the University of Kansas places a strong emphasis on equipping its graduates with the ability to proficiently comprehend intricate scientific research articles. To achieve this, the program mandates students to participate in a Selected Topics class, wherein they are organized into small groups. Within these groups, students systematically break down and explain different research articles to the class using structured PowerPoint presentations. To enhance the interactive nature of this activity, articles are thoughtfully chosen to mirror the chronological evolution of exemplary publications on a specific subject. In the 2023 spring semester, students delved into various instances of host immune regulation by microorganisms. A listing of the primary articles can be found in "A Primary (Sourced) Education" on page 12 of this issue, laying the groundwork for students to subsequently formulate an insightful review of a recently published article on the same topic. Enclosed herein are notable examples of papers authored by students enrolled in the course, serving as exemplars of those written by students enrolled in the course.

Partners in Crime:

Synergistic Anti-apoptotic Effects of HCMV Gene Products in Infected Cells

By Kaitlyn Sy

The means by which human cytomegalovirus (HCMV) evades the immune system has been a hot research topic for the past three decades. While much of the research has focused on viral proteins that interfere with the host's immune response, recent investigations suggest that proteins are not the only culprits. New findings by Hancock et al. indicate that HCMV-encoded microRNAs (miRNAs) work alongside viral proteins to produce synergistic antiapoptotic effects, shedding light on the mechanisms by which HCMV establishes latent infections in host cells.

Human cytomegalovirus (HCMV) is a common herpesvirus, estimated to infect over half the global population. In immunocompetent hosts, acute infection is controlled by a vigorous immune response, resulting in mild symptoms or no symptoms at all. However, in immunocompromised hosts, overwhelming replication of HCMV could lead to organ damage. Regardless of host immune status, HCMV establishes lifelong latent infection in CD34+ hematopoietic progenitor cells (HPCs). Latent infection may reactivate when the immune system is weakened, making HCMV the most common opportunistic infection in organ transplant patients.[1] HCMV infection is known to activate proapoptotic pathways that normally thwart viral replication,[2] so elucidating the mechanisms that enable HCMV to establish latency has been of interest to scientists. Understanding these mechanisms could aid in the development of more effective prevention and treatment strategies for HCMV infections.

Early research focused on interference of HCMV proteins with antigen presentation on MHC class I to evade detection by CD8+ T cells. HCMV protein US3 associates with and retains MHC class I molecules in the endoplasmic reticulum (ER).[3] Retained MHC class I molecules are then dislocated by the HCMV-encoded ER-resident transmembrane glycoprotein US11 to the cytosol to be degraded.[4] Cells that fail to express MHC class I are usually destroyed by natural killer (NK) cells, but UL18, an HCMV homolog of MHC class I heavy chain, generates surrogate MHC class I molecules by associating with β2-microglobulin and by binding peptides, and in so doing avoids NK-cell-mediated cellular cytotoxicity.[5] More recently, research has focused on direct interference of HCMV gene products with proapoptotic pathways. For example, HCMV protein vMIA suppresses cell death by binding to and sequestering the proapoptotic protein Bax, preventing mitochondrial membrane permeabilization, a key step in the intrinsic apoptotic pathway.[6] Another HCMV protein, pUL38, also inhibits the intrinsic apoptotic pathway by blocking proteolytic activation of two key apoptotic enzymes, caspase 3 and poly(ADP-ribose) polymerase (PARP).[7] Research about proteins that enable HCMV to establish latency has enabled scientists to piece together parts of the picture, but much remains elusive. However, work recently published by Hancock et al.2 uncovered three HCMV gene products—pUL7, miR-US5-1, and miR-UL112-3p—that modulate FOXO3a, a key transcription factor that targets several genes involved in both the intrinsic and extrinsic apoptosis pathways.[8]

pUL7, a glycoprotein secreted by HCMV-infected cells, binds directly to Fms-like tyrosine kinase 3 (Flt-3R), inducing downstream signaling cascades. While unnecessary for lytic replication, it is necessary for reactivation of HCMV from latency.[9] In a preliminary experiment, telomerized human fibroblasts (THFs) transfected with Flt-3R were stimulated with either pUL7 or Flt-3 ligand (Flt-3L, to serve as a positive control) with and without Flt-3R inhibitor AC220. Immunoblots indicated rapid phosphorylation of FOXO3a in THFs treated with pUL7 or Flt-3L and inhibition of phosphorylation in THFs treated with AC220, indicating that pUL7 stimulates phosphorylation of FOXO3a via a Flt-3R-dependent mechanism. To identify which pathway downstream of Flt-3R is involved in FOXO3a phosphorylation, the authors stimulated THFs with UL7 in the presence of inhibitors that block either PI3K or MEK. Only inhibition of MEK

prevented pUL-7-induced FOXO3a phosphorylation, suggesting that it occurs via the MAPK pathway. Phosphorylation of FOXO3a by pUL-7 was found to have an inactivating effect, indicated by significantly lower levels of BCL2L11 mRNA and BIM protein (both downstream products of FOXO3a target genes) in THFs stimulated with pUL-7, determined by qRT-PCR and immunoblot, respectively. However, stimulation with pUL-7 did not affect mRNA or total protein levels of FOXO3a. The authors' next question was whether phosphorylation of FOXO3a affects its distribution between the cytosol and the nucleus. Immunoblots and immunofluorescence microscopy indicated that FOXO3a is translocated to the cytoplasm in cells stimulated with pUL-7, and this translocation is inhibited when MEK is blocked. The authors repeated these experiments in RS4;11 cells (a lymphoblast cell line) and primary CD34+ HPCs, confirming that pUL7 has the same effects on FOXO3a across all three cell lines. Their data aligned well with reports of viral proteins that inactivate FOXO3a in other herpesviruses, but Hancock et al. did not stop with this. There was growing evidence in the literature that many viral proteins do not function alone, but rather work in tandem with microRNAs (miRNAs).[10] Bioinformatic analysis suggested that FOXO3a was a target of HCMV-encoded miR-US5-1 and miR-UL112-3p. In a preliminary experiment, Hancock et al. transfected HEK293T cells with vectors encoding miR-US5-1 or miR-UL112-3p. When FOXO3a mRNA and protein levels were assessed, there was a clear downregulation of both. Hancock et al. successfully obtained similar results when they repeated this experiment in primary CD34+ HPCs, confirming that miR-US5-1 and miR-UL112-3p target FOXO3a. Finally, to determine the effects of pUL7, miR-US5-1, and miR-UL112-3p on apoptosis, Hancock et al. transduced primary CD34+ HPCs with adenoviral vectors carrying pUL7, miR-US5-1, or miR-UL112-3p. When apoptosis was assessed via flow cytometry, it was observed that all three HCMV gene products protected cells from apoptosis (Illustrated in **Figure 1**).

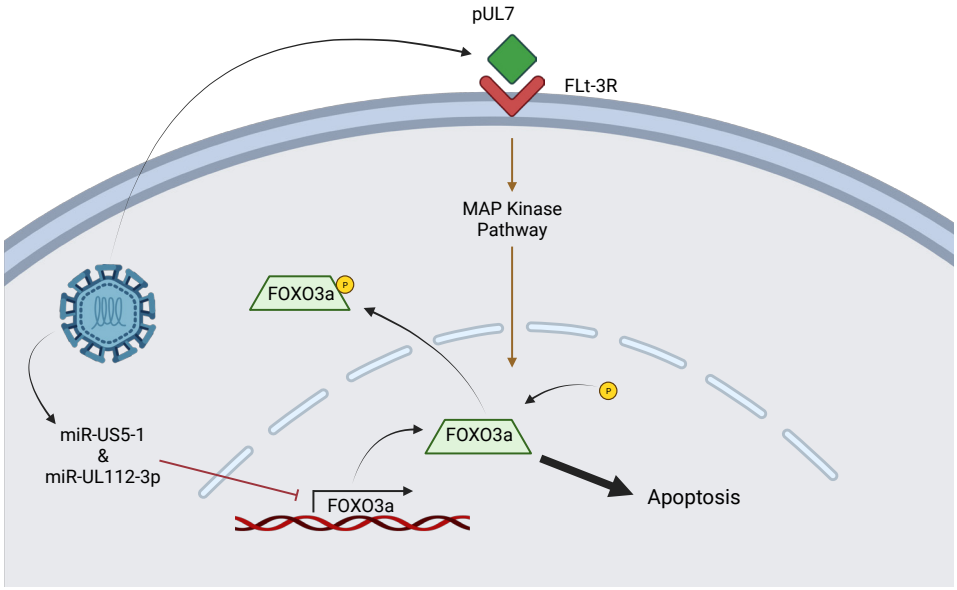

Figure 1 | Regulation of apoptosis.
The glycoprotein pUL7 and microRNAs collaborate to inhibit cellular apoptosis in HCMV-infected cells.

With this research in mind, several questions come to light. Are pUL7, miR-US5-1, and miR-UL112-3p expressed simultaneously in infected cells? If so, how is FOXO3a affected when the host cell expresses each of these products together, compared to separately? If not, what conditions warrant the expression of one (e.g., pUL7) versus another (e.g., miR-UL112-3p)? Even if these questions were not addressed by Hancock et al. in this study, their results shed light on an interesting synergy between antiapoptotic viral miRNAs and protein. miR-US5-1 and miR-UL112-3p target FOXO3a transcripts in HCMV-infected cells, significantly downregulating expression of FOXO3a. Further, any FOXO3a that host cells manage to produce is targeted by HCMV pUL7. Binding of pUL7 to Flt-3R triggers phosphorylation of FOXO3a via the MAPK pathway, resulting in translocation of FOXO3a from the nucleus to the cytoplasm. Consequently, the proapoptotic downstream products of FOXO3a gene targets are significantly downregulated, protecting host cells from apoptosis.

In conclusion, while understanding of the mechanisms by which HCMV establishes latency remains incomplete, this study by Hancock et al. adds valuable insights into the multifaceted approach by which HCMV lowers host defenses.

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A Sophie's Choice between Viral Survival and Immune Evasion

By Cinthia Moncada

The immune system has one important role: to protect the body from outside invaders. It consists of intercommunicating subsystems of innate and adaptive cells and intercellular pathways that allow the body to fight off disease-causing organisms. One way it does so is by producing interferons, which elicit antiviral responses. Type-1 Interferons (IFN-1, IFN-α, and IFN-β) are part of the innate immune response released by cells in response to pathogen engagement. Specifically, TANK-binding kinase 1 (TBK1) is known to induce IFN-1 production as part of the innate immune response triggered through the pattern recognition receptor, retinoic acid-inducible gene I (RIG-I).[1] When TBK1 is activated, it homo-dimerizes and phosphorylates interferon regulatory factor 3 (IRF3), which translocates from the cytosol to the nucleus, where it serves as a transactivator of IFN-1 expression.[2]

Viruses have evolved a number of mechanisms to suppress the immune system in order to replicate within its host. One example of such a virus is the herpes simplex virus type 1 (HSV-1). Previous studies have shown that the HSV-1 protein, UL46, is able to downregulate TBK1- dependent antiviral innate immunity though a direct interaction.[3] This prevents TBK1 homodimerization and subsequent phosphorylation of IRF3, culminating in a failure to produce IFN-1. A second example of a virus that interferes with the signaling pathway that produces IFN-1 is Human immunodeficiency virus -1 (HIV-1). HIV-1 consists of several accessory proteins that can suppress the innate immune response including the HIV-1 protease (PR). PR is an enzyme that cleaves viral polyproteins into functional subunits and also interferes with host cell proteins to suppress IFN production. PR accomplishes this by cleaving TBK1, the key signaling component of IFN production pathways discussed above.[2] Sundararaj et al. demonstrated the PR proteolytic-mediated cleavage of TBK1 and how the resulting cleavage products were unable to activate IRF3 thus reducing IFN-1 production (Illustrated in **Figure 1**).

To determine if HIV-1 PR could interact and cleave other kinases, Sundararaj et al. screened a library of 412 human kinases using the wheat germ cell-free production system. To determine the interaction of PR and these kinases, a cleavage activity assay was conducted. This assay identified the cleavage of TBK1 by a two-color immunoblot. Cleavage of TBK1 was detected by a recombinant TBK1 with an N-terminal FLAG tag and a C-terminal biotin tag. When probed with reagents bearing red (anti-FLAG-alexa592 antibody)

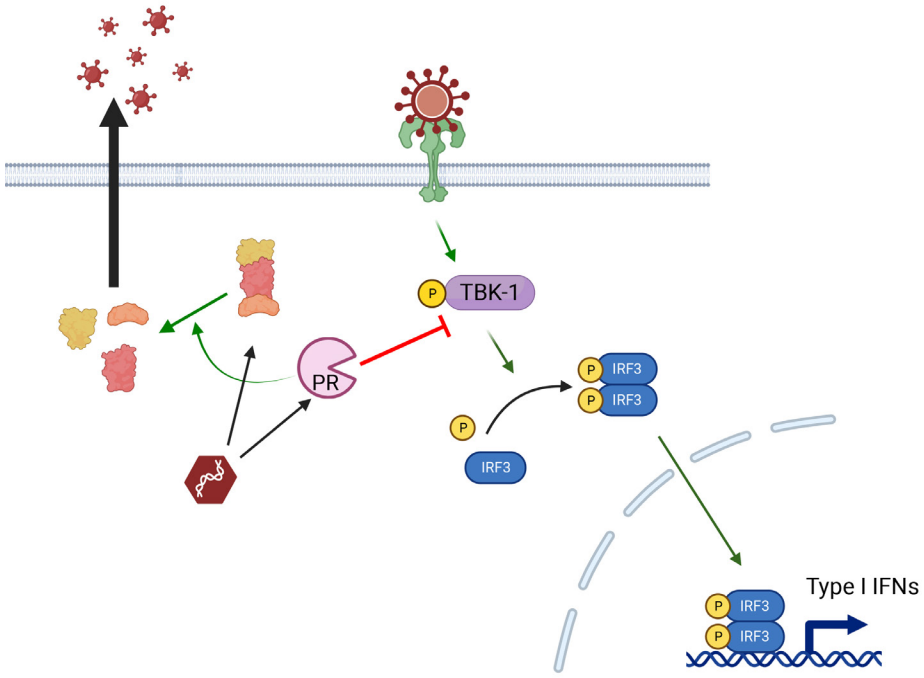

Figure 1 | Viral Protein Processing.
An HSV-1 viral genome synthesizes a protease to cleave its own polypeptide (left-side) critical to viral assembly and also interfere with interferon production mediated by TLR signaling (right-side).

or green (streptavidin-Alexa488) fluorochromes, intact molecules appeared yellow due to the proximity of the green and red fluorochromes binding intact TBK1, while cleaved TBK-1 produced two distinct bands, one labeling with the green fluorochrome, and one labeling with the red reagent. This demonstrated that HIV-1 PR could cleave TBK1 into the N-terminal green and C-terminal red fragments in vitro. PR D25N, a mutant of HIV-1 PR lacking proteolytic activity, demonstrated that the active site for viral and kinase proteolysis were the same. To verify that the cleavage of TBK1 by HIV-1 PR could occur intracellularly, HEK293 cells were co-transfected with HIV-1 Gag-Pol (Pol includes the protease) or a protease-negative HIV-1 Gag, with TBK1 in the presence or absence of the protease inhibitor, amprenavir. Gag-Pol, unlike Gag, was able to cleave TBK1 in the absence, but not presence of the inhibitor, indicating that TBK1 cleavage by HIV-1 PR occurs intracellularly.

Through amino acid sequencing, it was found that TBK1 was cleaved by HIV-1 PR between L683 and V684. From these data, TBK1 constructs with mutations at the cleavage site were generated. Western blot analysis of the WT TBK1 vs TBK1 mutants demonstrated that these amino acids were required for PR-mediated cleavage.

These experiments confirmed that HIV-PR cleaves TBK1, however, for this to be responsible for immunosuppression, it was necessary to show that cleaved TBK1 was incapable of activating the IFN-1 pathway. Therefore, HEK293 cells were co-transfected with IFNβ-promoter-Luc and either full length TBK1 or a cleaved TBK1 and monitored for luciferase activity. An increase in luciferase activity with full length TBK1, but not cleaved TBK1, was observed, demonstrating that cleaved TBK1 was unable to activate the IFNβ promoter. To further confirm that PR cleaved and inactivated TBK1, HEK293 cells were co-transfected with IFNβ-promoter-Luc, HIV-1 PR, wild-type (wt) TBK1 or a mutant (mt) TBK1. As predicted, wt, but not mtTBK1, -dependent luciferase activity was inhibited by the presence of HIV-1 PR. To determine whether HIV-1 PR inhibits both TBK1 and IRF3 phosphorylation, HEK293 cells were transfected with HIV-1 PR and TBK1, and a western blot for phosphorylated protein was conducted. In the presence of HIV-1 PR, TBK1 fails to become phosphorylated. Further, immunofluorescence was also used to demonstrate that IRF3 remained localized in the cytoplasm when PR was present.

Protease inhibitors (PIs) are commonly used to treat HIV-1, however due to widespread use, viral proteases have become resistant to these PIs. It was possible that PI-resistant PRs might lack the ability to effectively cleave host's proteins. To determine if this occurred, TBK1 were co-transfected in HEK293 cells with either drug resistant mutants of HIV-1 PR or wtHIV-1 PR , followed by western blot for TBK-1 cleavage. While wtHIV-PR was able to affectively cleave TBK1, the drug resistant mutants did not. Interestingly, mt PRs retained their ability to cleave the precursor viral protein, p55, into p41 and p24. This finding indicates that mutant PRs have altered host substrate specificity.

Sundararaj et al. show a mechanism where viral components can suppress innate immunity mediated by IFN-1, which is crucial for early control of a viral infection.[3] These findings give insight into how HIV-1 leverages its own reproductive strategy to suppresses immunity and how maintaining these basic functions as it co-evolves with antiviral medicines results in a choice between survival and becoming more vulnerable to immune clearance.[2]

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Beware the HIV Env, Which Comes to you in Glycosylated Coating

By Ambreen Niaman

Microorganisms have evolved enormously over the years which contributed to their immune evasion strategies. Some examples from Human Cytomegalovirus (HCMV) include the inhibition of major histocompatibility complex (MHC-1) via a family of homologous genes encoded on the HCMV genomic unique short (US) region.[1] This is achieved by the prevention of intracellular transport of MHC-1 molecules by an endoplasmic reticulum (ER) glycoprotein US3, which is an immediate early (IE) gene within the US region of the virus. The US11 gene, which encodes an ER resident type-1 transmembrane protein, and is expressed in later phases of infection, downregulates the MHC-1 expression by dislocating newly synthesized class-1 molecules from the ER to the cytosol where N-glycanase and the proteosome cause degradation of this complex.[2]

However, in the universe of immune evasion, the Human Immunodeficiency Virus–1(HIV-1) is king. By targeting CD-4+ Helper T Cells, the virus scores a masterful blow by effectively crippling the keystone of the host's immune system, eradicating a central player in supporting both cell-mediated and humoral immunity. This is further compounded by the nearly unlimited diversity afforded to an envelope protein presenting an external surface that functions primarily as a shield covering the more sequence-restricted receptor-binding elements.[3]

This review examines work by Montefiori, Robinson, and Mitchell on how HIV-1 uses protein glycosylation to dress as a wolf in sheep's clothing.

The envelope (Env) glycosylation process by HIV-1 can be used to distinguish intra-clade and inter-clade variation and provides insight into how glycosylation affects viral immunogenicity. The Env genes of this virus encode two heavily glycosylated proteins located in the viral membrane. Following expression, a large precursor protein (gp160) is formed, which is then cleaved into an amino-terminal outer membrane gp120, and carboxy-terminal derived gp41.[4]

Montefiori et al. posit that the extensive glycosylation of HIV-1 Env proteins,

gp120/gp41, plays an important role in immune evasion by covering the neutralization epitopes and presenting only the glycosylated portion of these proteins to the host immune system.

The importance of protein glycosylation in HIV-1 Env proteins assuming its ovine appearance was evaluated by selectively interrupting N-linked glycoprotein processing and assessing the subsequent replication, infectivity, and pathogenicity of the virus.[4]

One way in which the virulence of HIV results comes from CD4-gp120 interactions which induces T cell- T cell fusion events to form syncytia. Specifically, syncytia are produced by the interaction of gp120 or gp41expressed on an infected cell with the CD4 expressed on neighboring cell surfaces. The involvement of CD4 and gp120 interactions was demonstrated using anti-CD4 antibodies and site-directed mutation of gp120 to inhibit syncytia formation in vitro.

Further investigation revealed that it was specifically the N-glycosylation of envelope proteins that was necessary for HIV-1 to express its Env proteins intact and also to exert its cytopathic effects. Coincubation of HIV-infected H9 cells with uninfected MT-2 cells, and the N-glycosylation inhibitors castanospermine (a potent inhibitor of some glucosidase enzymes), 1-deoxynojirimycin (an alpha-glucosidase inhibitor), 1-deoxymannojirimycin (a mannosidase-I inhibitor), or tunicamycin (an inhibitor of GlcNAc phosphotransferase which acts early in glycoprotein synthesis.) was assessed. After 24 hours, the reduction in syncytial formation and cytopathic effect was observed. In contrast, no syncytium formation was observed when untreated H9/HTLV-IIIB cells were mixed with MT-2 cells preincubated with inhibitors. This suggested that HIV-induced syncytium formation and its associated cytopathic effects are greatly dependent on glycoprotein processing.

These results support a role for N-glycosylation of HIV-I Env proteins as necessary for cytopathic effects. About 50% of Env mass is contributed by host-cell derived N-linked glycans which are considered a major protective shield against immune recognition.[5] Because glycans are, in general, less amenable to inducing humoral immune responses, these can mask conserved polypeptide epitopes making it difficult for antibodies to recognize viable epitopes.[6]

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Chikungunya Virus Refuses to be Complemented

By Guenaele Raphael

Immune evasion is a hallmark of a pathogens' virulence and pathogenicity. To be successful in a host, pathogens have developed different strategies to overcome the immune system. For example, human cytomegalovirus (HCMV) inhibits MHC class I antigen presentation which prevents cytotoxic T cells from recognizing viral and self-antigens.[1,2] Other viruses, such as Hepatitis C (HCV), suppresses the immune system by blocking important signaling pathways involving pattern recognition receptors such as Toll-like receptors or RIG-I like receptors,[3,4] while Bordetella Bronchiseptica can inhibit the MAP kinase pathway and Nf-kB activation.[5]

Chikungunya virus (CHIKV), a re-emerging mosquito-borne pathogen, also successfully evades and suppresses the host immune response.[6] This virus was first isolated and discovered in Tanzania in the early 1950s,[7] and since then has caused major outbreaks around the globe. The most recent outbreaks occurred in 2013 within the regions of North and South America which prompted a concerted effort to better understand the pathogenesis of this virus, particularly how CHIKV interacts with the immune system. Prior studies have shown that CHIKV can elicit massive secretion of IFNs as well as proinflammatory chemokines and cytokines,[8] which help in controlling progression and dissemination of the pathogen inside the host. Interestingly, CHIKV has developed various strategies to counteract the host's immune system, including the disruption of IFN signaling

by the viral protease, nsP2, that can proteolytically cleave IFN.[9,10,11] Additionally, more recent research, from Nag et al., has shown an interaction between CHIKV and the human complement system.[12]

The complement system is made of a variety of plasma proteins that interact with one another to promote opsonization, neutralization, activation of phagocytosis, and other pro-inflammatory responses resulting in the assembly of the pore-forming, membrane attack complex (MAC). The complement system is activated through three distinct pathways, the classic pathway (CP), the lectin pathway (LP), and the alternative pathway (AP); each of which converge at the same effector molecule, C3 convertase. C3 convertase is an enzyme that can cleave a component of the complement system, C3 into C3a and C3b, which are a chemokine/mediator of inflammation and a potent opsonin, respectively.[12,13] This review article explores the work by Nag et al. that focuses on elucidating the mechanisms through which CHIKV can resist the human complement system by expression of a factor I-like activity.[12]

First, it was shown that CHIKV was able to activate the human complement system in a concentration-dependent manner. Briefly, a 2-fold serial dilution of sucrose-gradient-purified CHIKV was performed from 2.5 µg to 0.07 µg CHIKV and then incubated with normal human serum (NHS) for 45 min at 37ºC. Following the incubation, western blot analysis revealed that CHIKV was able to catalyze C3-to-C3a conversion between 0.15 µg and 0.31 µg CHIKV.[12] In a similar experiment,1.25 µg of CHIKV was incubated with NHS at different timepoints and showed time-dependent conversion of C3 to C3a from 5 minutes to 45 minutes. These results suggest that CHIKV is, in fact, able to activate the complement system in solution.

Since CHIKV demonstrated complement activation, the authors wanted to test if CHIKV is resistant to complement-mediated neutralization. To do so, complement-dependent neutralization assays were performed with the CHIKV. Briefly, CHIKV was incubated for 1h at 37ºC in different concentrations of NHS or heat-inactivated NHS (HI-NHS). The infectivity of these CHIKV samples were then determined by plaque assay on Vero cells. Compared to the virus-only control, incubations with high concentrations of NHS only reduced the number of plaques by 10 - 25%. To further substantiate this conclusion, a comparative analysis was performed with Chandipura virus (CHPV), a virus known for being sensitive to complement neutralization. As anticipated, CHPV exhibited a high sensitivity to complement-mediated-neutralization with a marked decrease in the number of plaques by up to 90%. These data further confirmed that CHIKV can resist complement-mediated-neutralization in vitro.

Recognizing the counterintuitive nature of CHIKV's ability to activate the complement system while also being relatively insensitive to complement-mediated neutralization, the authors sought to investigate if CHIKV was blocking complement-mediated neutralization by inhibiting deposition of complement components, specifically C3 and C4. Briefly, CHIKV samples, incubated with minimal essential media (MEM) or NHS at 37ºC for 1h, were ultracentrifuged and analyzed via western blotting to detect CHIKV proteins as well as C3 and C4. Both C3 and C4 were found to migrate with the viral fractions, suggesting that deposition of complement does in fact, occur. Analysis via electron microscopy further confirmed deposition of C3 and C4 components on CHIKV but in limited amounts which might be insufficient to trigger viral neutralization.

Since deposition of C3 and C4 on CHIKV does not trigger neutralization, the scientists decided to investigate the form of C3 that associates with the CHIKV virus. Normally, upon activation C3 is cleaved into C3a and C3b. However, this process can be abrogated by the presence of serine protease factor I, along with other cofactors such a factor H and CD46, forming the C3b inactivated form, iC3b; which occurs by the cleavage of the a-subunit of C3b. Interestingly, analysis via Western Blot reveals that the C3b component attached to the CHIKV virions lacks the a-subunit of C3b, suggesting the presence of a factor I-like activity by CHIKV. However, this factor I-like activity of CHIKV seemed to have no effect on C4b. Moreover, the degree of inactivation of C3b seems to be highly dependent on certain criteria: concentration of CHIKV, time of incubation, and the presence of the host cofactor H. Also, biochemical assays showed that cleavage of C3b is not due to the host factor I activity.

Overall, this paper suggests that CHIKV inhibits the activity of the complement system. These new findings offer valuable insights into the interaction of CHIKV and the complement system and contribute to its dissemination and progression in the bloodstream of infected hosts.

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A Primary (Sourced) Education

J.F. Treml*

I remember the first class where a professor said, while reviewing his syllabus, 'My primary goal for this class is for you to be able to read a scientific article.' Sitting in the classroom that day, I thought to myself, this is the dividing point. *Aha! I've just been learning well-worn biochemical pathways and never getting into any new material, but from now on, things are going to be different*! But things weren't different, because we still learned from the book and whenever I tried reading the literature, it was hopeless. I had several more classes that boldly stated the same goal only to result in the same outcome: disappointment and hopelessness.

Nevertheless, reading primary scientific journal articles is *the critical divide* when students become scientists; however, even when stated as a goal for a course, it is seldom explicitly taught.

This article provides a comprehensive review of one approach aimed at teaching undergraduate biotechnology students to effectively read primary articles. It further identifies common challenges faced by instructors and their students in obtaining and reading articles, including locating relevant articles, understanding technical terminology, comprehending complex methods, and navigating dense writing styles. Here, we outline an instructional strategy, which incorporates explicit instruction, modeling, and scaffolded support. We discuss potential pitfalls and evaluate the overall effectiveness of the approach. And finally, we present a curated list of articles used in the most recent iteration of our 'Topics in Biotechnology' class, serving as a resource for instructors interested in implementing a journal club class as part of an undergraduate program.

Scientific research plays a crucial role in advancing knowledge across all fields. Many journals still have titles referring to their origins, such as FEBS *Letters,* which evokes a time when scientists around the globe updated one another on their work by literally writing letters to one another.

Today, journal articles are fundamental to the research process, enabling scientists to stay informed about the latest discoveries, methodologies, and theories in their respective fields. Unfortunately, the explicit teaching of this skill is often non-existent in scientific training programs. This knowledge gap can pose challenges for young scientists, hindering their ability to keep up with advanced studies, stay updated on the latest research, and remain informed about developments in their field. It also leaves students ignorant of the rigor required to present their own work.

This article presents a review of one approach to teaching novice scientists to effectively read journal articles. It is worth noting that even experienced scientists, such as the instructors who teach these courses, had to learn this skill at one point in their careers. It is useful to adopt the beginner's mind in determining how to teach this skill.

One central challenge encountered by all scientists, students, and educators outside of the world's largest institutions, is *access*. Access to the full text of articles, including figures is essential to understanding and being able to reproduce data. In the absence of full text, following the development of ideas and locating important references is nearly impossible. The significance of searchable databases like the National Library of Medicine's PubMed notwithstanding, only full text can contextualize references fully. Fortunately, at least for research funded publicly by the US government, new rules are opening up access to articles.[1–3]

Once a major stumbling block, the availability of online search engines and reference tools has relegated the

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terminology barrier to a mere speed bump. Nevertheless, the complexity of novel methodologies and the density of scientific writing pose ongoing difficulties for budding scientists encountering these terms and concepts for the first time. This is precisely why journal clubs play an indispensable role in overcoming the steep learning curve and fostering lifelong learning environments. The need for hands-on, instructor engagement cannot be overstated.

Challenges in Identifying and Obtaining Articles

Scientists face numerous challenges when reading scientific articles. But before beginning, it is necessary to address two initial challenges, the first being students identifying and locating articles and the second, particularly important to smaller institutions wishing to implement this course, is full access to the articles themselves.

Identifying and locating articles is typically done using PubMed (**Figure 1**), a free, publicly maintained search engine available to anyone with an internet connection.

Beginning in 1879, The National Library of Medicine has published the Index Medicus as a monthly guide to medical articles in thousands of journals. This was initially only available *in print* as an array of tomes housed in subscribing libraries (also **Figure 1**). This eventually became digitally organized as the Medical Literature Analysis and Retrieval System (MEDLARS).[4] MEDLARS became publicly available in 1964, however in the absence of the internet, it was housed in the National Library of Medicine and only locally accessible. In 1971, the system first supported off-site access at medical libraries and was given the name 'MEDLARS Online' or 'MEDLINE' and could only be accessed indirectly through librarians using pre-programmed searches in order to not overwhelm the system, which was capable of supporting up to 25 simultaneous users.[5] In 1996, as home computers were becoming capable of interacting with the internet via web browsers, the system was publicly launched online as PubMed, which contains the entire Indicus Medicus, as well as referencing additional databases.

The search engine of PubMed only addresses the 'identification' problem but does not provide access to the articles beyond abstract viewing. The papers themselves often lie behind paywalls making regular access virtually impossible to any but large, research institutions.

In August of 2022, the United States White House Office of Science and Technology Policy updated a 2013 memorandum that increased free access to publications of research resulting from the expenditure of public funds. The 2008 public policy had allowed for a 12-month embargo from public access, while the 2022 action moved "[t]o promote equity and advance the work of restoring the public's trust in Government science, and to advance American scientific leadership[by] amend[ing] federal policy to deliver immediate public access to federally funded research."[1,6,7] The goal for full, free access to all publicly-funded research immediately following publication is expected to be fully realized by 2025.[2]

In the meantime, older publications are increasingly becoming available through Google Scholar or are often

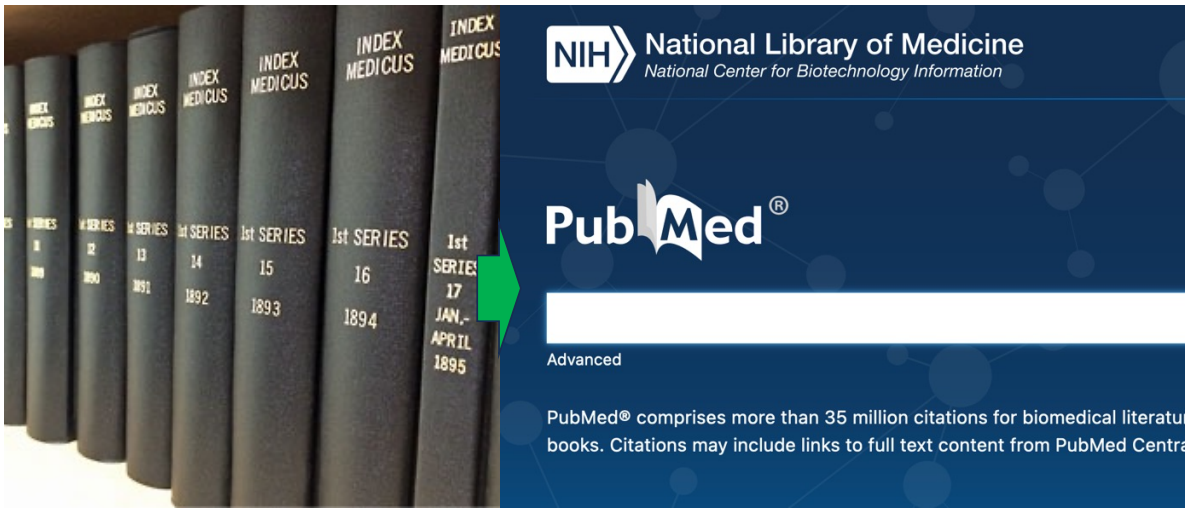

Figure 1 | From Index Medicus to Pubmed. http://pubmed.ncbi.nlm.nih.gov

obtained via legally questionable sources such as ResearchGate. In the absence of any other means, another method is to directly contact the corresponding author and simply ask for a copy of the article.

Explicit Instruction & Modeling

Our 'Selected Topics in Biotechnology,' encourages collaboration between first-and second-year students (Juniors and Seniors, respectively). The experience of second-year students, combined with instructor-led sessions, guides first-year students through the reading process. These sessions involve detailed explanations of figures, experimental procedures, and results. Reading and comprehending articles prior to working with students can aid instructors in effectively guiding discussions. However, it is also useful to not thoroughly digest every detail prior to group meetings in order to model information processing in front of the group. This approach allows students to witness and take part in, the collective effort required to understand scientific concepts, explore references, critically analyze experiments, and question potentially ambiguous results or misleading interpretations.

While the instructor initially plays a significant role in interpreting the paper, it is important for undergraduate students to navigate the article independently to build their own understanding. As part of this process, student groups are encouraged to explore and explain new techniques within the context of their presentations to the class.

A method of scaffolded assistance is employed to prevent students from simply relying on the professor to do the intellectual work for them. This involves providing students with temporary support aimed at transferring responsibility to students as they as they gain proficiency.[8] This can involve providing students with a cartoon summary of the system, asking students to fill in the elements they understand, sketching cell-cell interactions with relevant receptors engaged, outlining the logical series of experiments performed, forming a list of key terms, and asking students to connect the main ideas in the article. With each interaction with students, scaffolding should be reduced as students take on more of these tasks themselves.

Explicit instruction is an instructional approach that is characterized by clear and direct teaching methods, including the use of modeling, coaching and guided practice. This can involve modeling how to read the abstract, introduction, methods, results, and discussion sections of scientific articles for students by verbally walking through the process with a small group. Although there are numerous ways to approach a primary research publication, the method employed in our course is very hands-on.

The course is structured in a way that establishes groups of students (preferably randomly or semi-randomly) that include one or more first-year students and one or more second year students (the program spans the junior and senior year of students' undergraduate term). Because we require students to take this course twice, second-year students have experience from their previous year and help guide first-years through the process.

Each year, I as the instructor, present the first article as one-part presentation instruction and one-part model presentation. Students are provided with a template for a presentation and encouraged to use it, while I present both the content of the article and commentary on why I'm presenting the way that I am. It's important to keep this article short and simple because I also go over the syllabus and course requirements during the same one-hour class time.

In preparation for subsequent presentations, each group is required to schedule at least one meeting of about two hours with the instructor prior to presenting to the class. Although initially established as Q&A sessions to clarify any issues with complicated figures or interpretations, over the years, these quickly transformed into a figure-by-figure explanations of how each experiment was done and what the results revealed. If extra time is needed, these meetings are repeated until the entire paper is covered.

This models an approach to reading the articles by explaining the structure of a scientific article, identifying key concepts, and highlighting the use of technical terminology. Breaking down an article with students presents examples of effective reading strategies through involving them with the instructor's thinking. It is helpful to read through and understand an article before approaching it with students, but I often do not examine the details prior to my meeting so I can organically process the information in front of the group. Benefits to this may include, allowing students to see that everyone must work to understand the science, to demonstrate how to follow

references while working out the problem, to openly inquire about the purpose of each experiment within a paper, and to question results that may be ambiguous or interpretations that are misleading.

Although the instructor does much of the initial work of interpreting the paper, at the undergraduate level, it is entirely appropriate as students will need to re-navigate the paper in building their presentation in a way that they can later explain to the rest of the class. As part of the process, groups are additionally asked to investigate new techniques when they occur and explain them to the class within the context of their presentations.

Even with this level of modeling and explicit instruction, students invariably find the workload to be heavy and intellectually rigorous.

Conclusion

With this course, I too boldly state that the objective of the course is to equip students with the skills to read journal articles. Hopefully, this course comes closer to meeting that goal than the legion of courses I heard that goal announced in.

Out of all the classes I teach, none has received as much positive feedback from students as my 'Topics' class. Many students have returned with comments such as, "This was the class that transformed me into a scientist" or "This was the first class that actually helped me comprehend the process of scientific inquiry." While it is important to remember that anecdotes do not constitute solid evidence, these are the responses that motivate me to continue teaching this course year after year, firmly believing that it genuinely makes a meaningful impact.

Reading List – Immune Evasion

Pages 13 and 14 contain the list of articles used in the most recent iteration of our course. This is the same course that established the background knowledge students had prior to selecting recent articles on immune evasion to base their review articles from the preceding section of this journal. The bold articles were assigned for presentation, while other articles were optional, supportive readings. When more than one paper within a topic is presented, "(class #)" is used to distinguish which paper was presented in each class. These were presented by different groups of students.

Future volumes of this journal will include the reading lists for this class (without an accompanying article). I encourage other educators to take and use these lists, in whole, or in part, in your own courses.

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Selected Topics

Immune Evasion by Microbial Pathogens Reading List

1. Review of Immune Evasion/MHC Presentation

a. **Ahn, K. et al. Human cytomegalovirus inhibits antigen presentation by a sequential multistep process. Proceedings of the National Academy of Sciences 93, 10990–10995 (1996).**

b. Finlay, B. B. & McFadden, G. Anti-Immunology: Evasion of the Host Immune System by Bacterial and Viral Pathogens. Cell 124, 767–782 (2006).

c. Ploegh, H. L. Viral Strategies of Immune Evasion. Science 280, 248–253 (1998).

2. Antimicrobial peptides: Innate immunity effectors

a. **Legarda, D., Klein-Patel, M. E., Yim, S., Yuk, M. H., and Diamond, G. "Suppression of NF-kappa B-mediated Beta-defensin Gene Expression in the Mammalian Airway by the Bordetella Type III Secretion System." Cellular Microbiology 7 (2005): 489-497.**

3. Toll Like Receptors

a. **Li, K. et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. Proceedings of the National Academy of Sciences 102, 2992–2997 (2005). (class 1)**

b**. Meylan, E., Curran, J., Hofmann, K. et al. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. Nature 437, 1167–1172 (2005). https://doi.org/10.1038/nature 04193. (class 2)**

c. Tan, Y., Zanoni, I., Cullen, T. W., Goodman, A. L. & Kagan, J. C. Mechanisms of Toll-like Receptor 4 Endocytosis Reveal a Common Immune-Evasion Strategy Used by Pathogenic and Commensal Bacteria. Immunity 43, 909–922 (2015).

4. TAP

a. **Tan, Y., Zanoni, I., Cullen, T. W., Goodman, A. L. & Kagan, J. C. Mechanisms of Toll-like Receptor 4 Endocytosis Reveal a Common Immune-Evasion Strategy Used by Pathogenic and Commensal Bacteria. Immunity 43, 909–922 (2015).**

b. van de Weijer, M. L., Luteijn, R. D. & Wiertz, E. J. H. J. Viral immune evasion: Lessons in MHC class I antigen presentation. Seminars in Immunology 27, 125–137 (2015).

5. MHC Class I Antigen Presentation

a. **Wiertz, E., Jones, T. R., Sun, L., Bogyo, M., Geuze, H. J., and Ploegh, H. L. "The Human Cytomegalovirus US11 Gene Product Dislocates MHC Class I Heavy Chains from the Endoplasmic Reticulum to the Cytosol." Cell 84 (1996): 769-779. (class 1)**

b. Ye, Y. H., Shibata, Y., Yun, C., Ron, D., and Rapoport, T. A. "A Membrane Protein Complex Mediates Retro-translocation from the ER Lumen into the Cytosol." Nature 429 (2004): 841-847.

c. **Neumann, L., Kraas, W., Uebel, S., Jung, G., and Tampe, R. "The Active Domain of the Herpes Simplex Virus Protein ICP47: A Potent Inhibitor of the Transporter Associated with Antigen Processing (TAP)." Journal of Molecular Biology 272 (1997): 484-492. (class 2)**

6. MHC Class II antigen presentation

a. **Pancio, H. A., Vander Heyden, N., Kosuri, K., Cresswell, P., and Ratner, L. "Interaction of Human Immunodeficiency Virus Type 2 Vpx and Invariant Chain." Journal of Virology 74 (2000): 6168-6172. (class 1)**

b. Zuo J, Rowe M. Herpesviruses placating the unwilling host: manipulation of the MHC class II antigen presentation pathway. Viruses. 2012 Aug;4(8):1335-53. doi: 10.3390/v4081335. Epub 2012Aug 22. PMID: 23012630; PMCID: PMC3446767

c. **Stumptner-Cuvelette P, Morchoisne S, Dugast M, Le Gall S, Raposo G, Schwartz O, Benaroch P. HIV- 1 Nef impairs MHC class II antigen presentation and surface expression. Proc Natl Acad Sci U S A. 2001 Oct 9;98(21):12144-9. (class 2)**

7. The Proteasome and ubiquitin

a. Zhou, H. L., Monack, U. M., Kayagaki, N., Wertz, I., Yin, J. P., Wolf, B., and Dixit, V. M. "Yersinia Virulence Factor YopJ acts as a Deubiquitinase to Inhibit NF-kappa B Activation." Journal of Experimental Medicine 202 (2005): 1327-1332.

8. Programmed cell death

a. **Everett, H., Barry, M., Lee, S. F., Sun, X. J., Graham, K., Stone, J., Bleackley, R. C., and McFadden, G."M11L: A Novel Mitochondria-localized Protein of Myxoma Virus that Blocks Apoptosis of Infected Leukocytes." Journal of Experimental Medicine 191 (2000): 1487-1498. (class 1)**

b. **Henderson, S., Rowe, M., Gregory, C., Croomcarter, D., Wang, F., Longnecker, R., Kieff, E., and Rickinson, A. " Induction of Bcl-2 Expression by Epstein-Barr-Virus Latent Membrane Protein-1 Protects Infected B-cells from Programmed Cell-death." Cell 65 (1991): 1107-1115. (class 2)**

9. Molecular Mimicry

a. **Reyburn, H. T., Mandelboim, O., ValesGomez, M., Davis, D. M., Pazmany, L., and Strominger, J. L. "The Class I MHC Homologue of Human Cytomegalovirus inhibits attack by Natural Killer Cells." Nature 386 (1997): 514-517.**

10. Cytokines

a. **Kledal, T. N., Rosenkilde, M. M., Coulin, F., Simmons, G., Johnsen, A. H., Alouani, S., Power, C. A., Luttichau, H. R., Gerstoft, J., Clapham, P. R., ClarkLewis, I., Wells, T. N. C., and Schwartz, T. W. "A Broad-spectrum Chemokine Antagonist Encoded by Kaposi's Sarcoma-associated Herpesvirus." Science 277 (1997): 1656-1659.**

KU Biotechnology Undergraduate Research

Often times undergraduate science students' laboratory exposures are limited to single-day (or otherwise encapsulated) experiences. It is common for students to be given comprehensive, stepwise protocols that provide little encouragement to fully understand why each step is performed. When the experiment is completed and a lab report is turned in, students are left with only a vague understanding of whether their lab 'worked' or not, but don't understand why, nor are they offered the opportunity to revisit any of their work.

This lack of follow-through fails to develop the student's ability to think critically about the skills and laboratory techniques they are expected to learn. Recent pedagogy touts that 'authentic undergraduate research experiences' are the cure for these cookie-cutter labs. However, the definition of an 'authentic experience', or how to provide one, remains elusive.

In the KU Edwards Campus Biotechnology program, we have developed a senior capstone research curriculum based on the experience of a graduate student working on their independent research topic. The curriculum consists of one semester of research and planning and one of hands-on experimental work. Students independently conduct a research project of their choice and gain exposure to valuable aspects of scientific research such as budgeting and inventorying their own reagents, scheduling with Gantt charts, and troubleshooting.
Throughout the year, weekly lab meetings are designed to keep students moving forward and give them practice in presenting updates on their work. In students' own words, these meetings cultivate an environment where students are inspired by and invested in not only their own capstone projects but those of their classmates as well.

While students do not always achieve their initial objectives, the process of working through problems and dealing with unexpected results provides what we believe is the 'authentic undergraduate research experience' that so many have called for.

Analysis of the Antioxidant & Antimutagenic Potential of CGA, a phenolic component of Coffee, in Inhibiting Mutations in *Salmonella typhimurium*

Ambreen Niaman*, J.F. Treml*, and R. Logan*†

Excess cellular radical oxygen species are associated with an increase in cellular, including genetic damage. In somatic cells, this may lead to cancer when genes associated with cell growth and reproduction are impacted. Opposing radical oxygen species are antioxidants which can quench free radicals that otherwise may damage a cell's DNA. The body produces some antioxidants naturally, such as glutathione, while others are obtained through diet, including vitamins C and E. Chlorogenic Acid (CGA) is one example of a polyphenolic compound in coffee with the potential to exhibit antioxidant and antimutagenic properties. The concentration of CGA in a commercially available coffee was measured and its potential to exhibit antioxidant properties was assessed. An extract was prepared in which the spectral properties of CGA were exploited to quantitate its concentration in green coffee extract via HPLC. The antioxidant activity of CGA was characterized by measuring its relative ability to neutralize free radicals using a colorimetric assay vs. a pure vitamin C standard. The antimutagenic activity of CGA was to be assessed via an Ames test using mutant Salmonella strains incapable of producing the amino acid histidine. The results demonstrated a considerable amount of CGA in green coffee extract, that exhibited antioxidant properties. Future work will assess antimutagenic effects of CGA in the extract compared to the antimutagenic effect of vitamin C and pure CGA standards. Overall, these results suggested that coffee beverages may serve as potent antioxidants with the potential to protect consumers from the harmful effects of mutagenic free radicals.

Coffee is a beverage, prepared as an extract from roasted coffee beans, widely consumed across the world. It has been regarded as an ancient wonder drug due to the presence of a variety of Phyto biomolecules with therapeutic potential1. Seeds of the coffee plants are separated and dried to produce green coffee beans which are then roasted and ground into fine granules that are boiled and steeped in hot water.[1,2,3] Many of the polyphenols in coffee are also found in other plant-based foods. These polyphenols are believed to have antioxidant as well as antimutagenic properties.[4] The antioxidant property of polyphenols[5] is exhibited by their ability to neutralize reactive oxygen species(ROS) in the body which cause oxidative stress in normal, healthy cells by damaging their constituent proteins, lipids, and DNA and subsequently leading to oncogenesis.[6,7] Thus, these compounds provide health benefits to people who consume these foods on daily basis.[8]

Reactive oxygen species (ROS) are associated with an increase in cellular, including genetic damage. These are highly reactive chemicals formed from diatomic oxygen O_2 such as peroxides, superoxide, and hydroxyl radicals.[5] ROS's are produced endogenously by some biochemical reactions occurring during respiration and photosynthesis inside mitochondria, peroxisomes, and chloroplasts.[5] Whereas exogenous sources include pollution, smoke, drugs, xenobiotics, microplastics, and radiation.[5]

Chlorogenic acid (CGA), one of the polyphenolic compounds in coffee, is claimed to exhibit antioxidant properties.[9] This naturally occurring compound is found in many plant-based foods, including coffee.[10] Its concentration in coffee is documented to be highest in Arabica coffee beans which are the most popular type of beans used worldwide.[2,8]

Coffee made with green coffee beans serves as a dietary vehicle for polyphenolic compounds such as CGA which exert their antioxidant activity on cells, protecting them from free radical attacks and preventing mutations potentially leading to cancers.[7]

While we know that CGA may be beneficial, we do not know to what extent it exerts its effect at the cellular level. Thus, in vitro studies were performed to evaluate the antioxidant and antimutagenic potency of CGA and green coffee extract.[11] Several experimental questions were explored regarding CGA such as the concentration of CGA in commercially available green coffee beans, its ability to neutralize free radicals, and subsequently preventing the mutation in healthy cells.[12]

Overall, this research project seeks to identify compounds in nature that can prevent cancers in humans by neutralizing free radicals in the body.[13] This research may serve to establish a therapeutic use for these compounds that could be incorporated as a preventive measure to reduce the probability of cancer development.[13,14]

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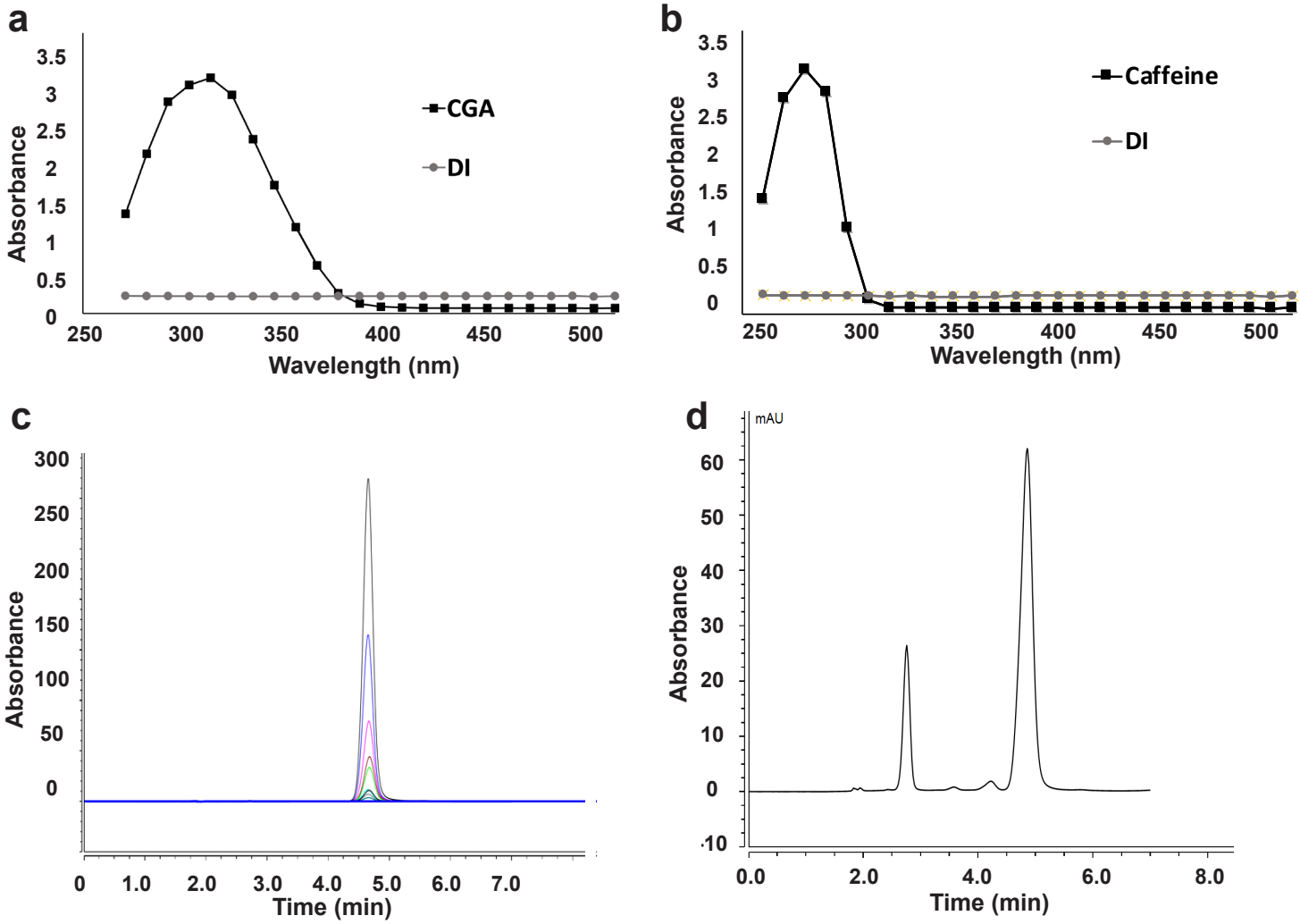

Figure 1 | Quantification of Chlorogenic acid (CGA) via Spectrophotometer and HPLC. CGA standards were prepared in DI matrix with organic crash.
(**a**) Absorbance Spectrum of 70.6μM Chlorogenic acid between 270 and 500nm. (**b**) Absorbance spectrum of 0.32mM Caffeine between 250 and 400nm. (**c**) Overlay of various concentrations of CGA ranging from 250 μM to 0.975 μM were run on HPLC at 20% B (Methanol + 0.1% Formic acid) + 80% A (DI + 0.1% Formic acid) on a reverse-phase, BDS Hypersil C 18 column. (**d**) CGA Calibration curve for HPLC Method.

Materials & Methods

Generation of the Green Coffee Bean Extract

Coffee was extracted by grinding the green coffee beans (Unroasted Green Coffee Beans, Primos Coffee Company) into a medium-ground powder. in order to optimize the extraction of the polar CGA molecule, the ground powder was weighed & boiled in deionized (DI) water at 94°C for 5 minutes for maximum extraction. DI water was used because of its potential as an efficient solvent for the extraction of polar molecules as well as its correlation to human consumption. The mixture was filtered using a Q8 filter paper (Fisher Scientific; Cat No: 09-790-B). The filtrate was stored at 4°C.

Quantification of CGA

The quantification of CGA in green coffee bean extract was carried out using "High Performance Liquid Chromatography" (HPLC). The CGA filtrate was analyzed using a C-18 column (Thermo-Scientific Cat No.28105-154630) and was run at 20% B [Methanol(Fisher Scientific: Cat # A452-4) + 0.1% Formic acid(Honeywell Fluka: Cat # 94318)] for 7 minutes using an isocratic method. The absorbance spectrum of standard CGA(Sigma Aldrich: C3878-1G) was run on a spectrophotometer (SpectraMax M3) and an optimal detection wavelength of 310 nm was determined. A standard curve was generated on the HPLC using various concentrations of pure CGA with 20% B. Green coffee extract was run on the HPLC & its average area under the curve (peak) was substituted into the equation derived to fit the standard curve. CGA concentration was determined by the following equation:

[CGAmM]=(OD310nm-1.2213)/0.2492

Assessment of Antioxidant Activity

A colorimetric assay using an ABTS (Cat No: KF01002) kit was performed using a 1:30 concentration of reagents A & B. A bar graph was generated depicting the antioxidant activity exhibited by DI water, Vitamin C (Ascorbic acid, Sigma Aldrich: 47863), CGA(Sigma Aldrich: C3878-1G), and coffee extract. DI was used as a negative control while Vitamin C (Ascorbic acid), being a well-known potent antioxidant, was used as a positive control. The antioxidant potential of the coffee extract was established by the equation:

Antioxidant potency = 1/(Abs 734nm)

Verification of rfa mutation in Salmonella strains

CGA's potential to block mutation in cells caused by free radicals was to be assessed via the Ames test. For this purpose, mutant (His-) strains of Salmonella typhi TA 1535 (Cat No: 29629) & TA 1538(Cat No: 29631) were used. The rfa mutation in these strains was confirmed via a crystal violet sensitivity test. For this purpose, 520 mg of nutrient broth (MP Biomedicals: Cat # 1007917) was mixed in 40 ml of sterile DI water. This broth was divided in half and dispensed to two sterile 50 ml Erlenmeyer flasks and labeled as TA 1535 & TA 1538. Then, using aseptic technique, both strains were inoculated into the Erlenmeyer flasks containing the broth. Those flasks were secured with wax tape and incubated for (10-12 hours) at 37 C° & 120 rpm in an incubator (ThermoScientific: Cat # SHKE4000). Nutrient agar plates were made by dissolving 7.5 g of nutrient agar (Fisher Scientific: Cat # S71614A) in 500 ml of sterile DI water. This mixture was autoclaved at 121 C° for 20 minutes and plates were poured. 100 µL of a fresh culture of each strain was added onto plates. 0.1% Crystal Violet solution was prepared by dissolving 1mg of Crystal Violet powder (Millipore Sigma: Cat # C6158) in 1 ml sterile DI water. The sterile paper disks 6mm in diameter (Becton Dickinson & Company: Cat # 231039) were soaked in 100 µL of this 0.1% Crystal violet solution and ~ 5 discs were placed on Nutrient agar plates labeled with both strains. These plates were incubated at 37 C° for 24 hours.

Results

Green Coffee Extraction and determination of CGA content

Green coffee extract was made by heating ground coffee beans in DI to 94 C° for 5 minutes and then steeping for 24 hours. The absorbance spectrum of pure CGA was measured from 250-750 nm at 10 nm intervals on the Spectrophotometer. The wavelength at which pure CGA exhibited the highest absorbance of light was selected as the optimum for detecting CGA in the coffee extract (**Figure1a**). A set of CGA standards was prepared and mixed 1:2 with methanol. CGA standards ranging from 0.975 µM to 250 µM were run in triplicates on the HPLC and detected by absorbance of light at 310 nm. Chromatograms of the standards were superimposed (**Figure 1c**, 250, 125, and 62.5µM results are highlighted). Using the average areas under the curve of these standards, a standard curve was created (data not shown). Coffee samples were similarly prepared with different dilutions in a 1:2 methanol mixture and run on the HPLC column. The chromatogram revealed two major peaks (**Figure 1d**). The peak representing CGA was confirmed by making a superimposed image of the standards and coffee extract (data not shown). We briefly considered the possibility that the smaller peak appearing on the chromatogram could be caffeine, but this was ruled out after the absorption spectrum of caffeine demonstrated no absorption at 310nm (**Figure 1b**). The concentration of CGA in the diluted coffee sample was calculated to be 57.7µM by using the linear equation shown in (**Figure 1d**). The CGA concentration in the undiluted coffee extract was calculated as 769 µM or 272.5 mg/L.

The Antioxidant Assessment of Green Coffee Bean Extract

The antioxidant activity of CGA was determined by a colorimetric assay using an ABTS kit. The results reveal that pure CGA has less antioxidant activity compared to

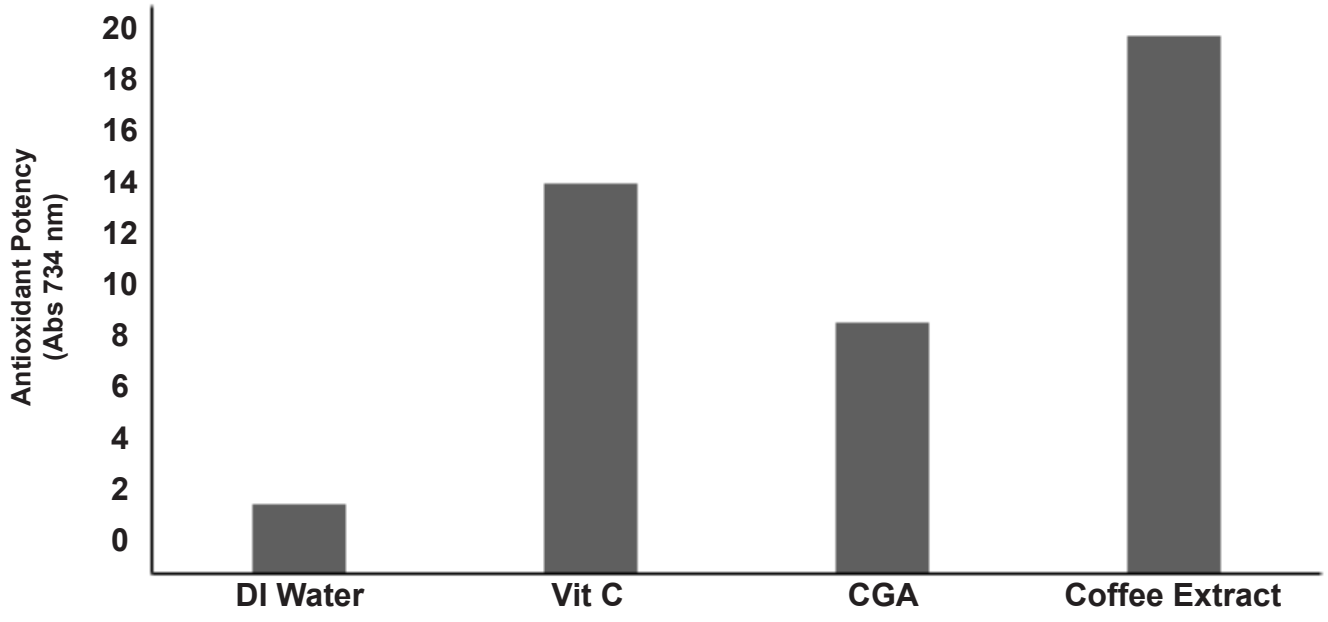

Figure 2 | Comparing the antioxidant potency of Green Coffee bean extract to the standard Vitamin C (Ascorbic acid) & Pure CGA.

Five µL of Vitamin C (769 µM), pure CGA (769 µM), Coffee extract and DI Water were added to 96-well plate. Then, 200 µL of colorimetric substrate (A & B) in the ratio of 1:30 was added to each well and incubated for 5 minutes at 27°C. Absorbance measured at 734 nm using spectrophotometer.

the known antioxidant Vitamin C. However, the green coffee bean extract exhibited almost double the antioxidant activity of Pure CGA when normalized for molar concentration (**Figure 2**). This suggests the presence of additional, possibly phenolic, compounds present in the coffee that could be exerting antioxidant effects independent of CGA.

Determination of Antimutagenic property of CGA

The question of whether CGA in green coffee beans exhibits antimutagenic effects was to be determined using mutant bacterial strains. In preparation to execute Ames tests[15] on these compounds, histidine mutations in each of two mutant strains Salmonella (TA 1535 & 1538) were verified by a crystal violet sensitivity test. Salmonella TA 1535 has a missense mutation, while TA1538 has a frameshift mutation. Additionally, both strains exhibit a rfa mutation in genes responsible for LPS synthesis that are disrupted leading to truncated LPS formation.[16] This mutation serves as a marker to ensure that these bacteria cultures have not been overgrown by wild, or other lab strains of bacteria. Crystal violet, being a large molecule was able to penetrate the disrupted LPS-containing membranes and lysed those cells. The crystal violet sensitivity test revealed that both strains were equally sensitive to the compound.[17] This is clearly visible on the plates exhibited by zones of inhibition around the discs soaked in crystal violet solution (**Figure 3**).

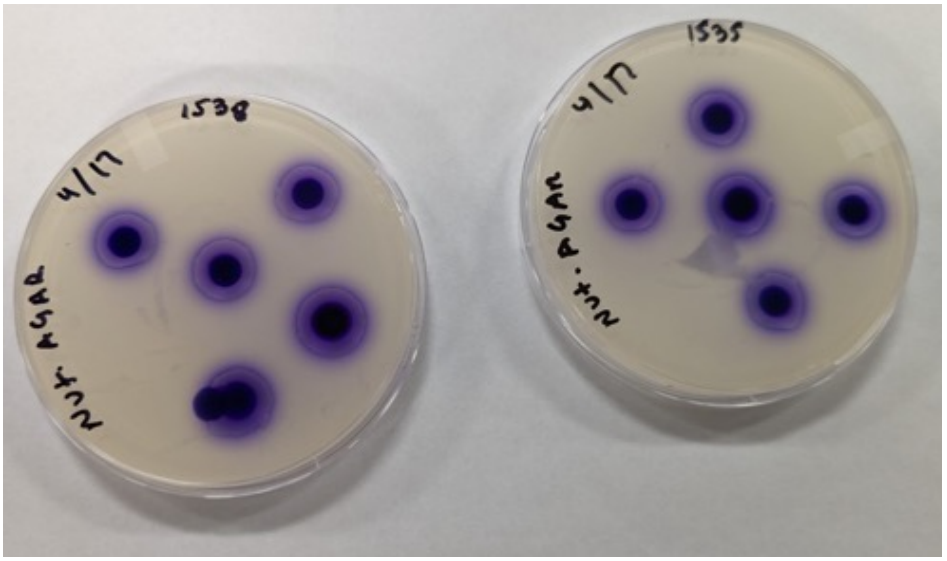

Figure 3 | Crystal Violet Sensitivity Test.
Salmonella typhimurium strains TA 1535 & TA 1538 exposed to 0.1% Crystal Violet solution (1 mg/ml) using 6mm sterile paper disks (Disk diffusion method). Zones of inhibition observed after incubation at 37° C for 12 hours, confirming rfa mutation.

Discussion

Unroasted Arabica green coffee beans were obtained from a Nicaraguan source. These mutant/hybrid arabica coffee beans originated from the combination of Maragogype (known as "elephant beans") and Catura beans, famous for excellent flavor.[18] These "Maracatu" hybrids have CGA concentrations calculated to be 272.5 mg/L which is relatively lower compared to the concentration in unroasted raw coffee beans as examined by Awwad et al.[19] On the other hand, they possess CGA concentrations comparable to those measured in light-roasted beans identified in the same study. The variation in CGA concentration might be due to the difference in extract preparation or variable storage conditions. It could also be due to the fact that these mutant beans are derived from the combination of other varieties, which might contribute towards the lower concentration of CGA.

The hypothesis that CGA in green coffee beans exerts its antioxidant effect was supported by their ability to neutralize free radicals in the colorimetric assay.[20,21] According to Tang et al., there was a strong correlation between antioxidant capacity and total phenolic compounds in Pitahaya fruit peel, which highlighted the contribution of each phenolic compound to the overall antioxidant capacity.[22] Similar results were obtained when the green coffee bean extract was exposed to free radicals in a colorimetric assay. According to Figure 2, the antioxidant capacity exhibited by the extract was almost double the antioxidant potency of Vitamin C and pure CGA on a molar basis. These results suggest the copresence of other compounds in coffee beans besides CGA, which might contribute towards the total antioxidant capacity exerted by the extract.

The crystal violet sensitivity test revealed that two Salmonella strains (TA 1535 & 1538) possess rfa mutation which renders them sensitive to penetration of large molecules. Crystal violet, being a large molecule and highly toxic, kills the cells causing the appearance of zones of inhibition around each disc soaked in it.

Future Directions

Future direction includes evaluating the antimutagenic potential exhibited by CGA via Ames test. The mutant strains (TA 1535 & 1538) don't have the ability to grow on media lacking histidine because of missense and frameshift mutations in their genome, respectively. These strains, when exposed to mutagens would grow on media without histidine confirming the reversion of mutation. Conversely, CGA and Vitamin C would be expected to prevent this reversion and spontaneous colonies won't be observed. Prevention of mutation is a promising aspect of utilizing coffee as a therapeutic for preventing cancer and opens a possibility for CGA to be considered as a potential cancer-preventive drug.

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Author's Biography

Ambreen Niaman is a senior in Biotechnology BAS program at KU Edwards Campus. She received an associate degree in Biotechnology from the Johnson County community college. She plans to graduate

in Spring 2023 and is looking forward to work in the science industry to expand her knowledge and experience.

Author's Contributions

A.N. contributed to the design, experimental work, and writing of this work; J.F.T. contributed to the editing and design of this work; R.L.L. contributed to the editing and design of this work.

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Cell Culture Media Optimization for Increased Production of Recombinant Proteins in Insect Cells

Guenaele Raphael*, R. Logan*, and J.F. Treml*†

The insect cell/baculovirus expression system (IC-BEVS) has been at the forefront of biotechnological research, and has served as a tool to produce several human therapeutics such as Cervavix ® (preventative vaccine against the human papilloma Virus), Flublok ® (preventative vaccine against seasonal influenza virus), Provenge ® (therapeutic treatment against prostate cancer) and Glybera ® (gene therapy treatment for lipoprotein lipase deficiency). IC-BEVS is an attractive alternative to mammalian cells for biomanufacturing as it offers advantages such as easy adaptation to serum-free media, high levels of protein expression and post-translational modifications, and is appropriately scalable for manufacturing 2. Despite these advantages, the use of IC-BEVS to produce recombinant proteins can be costly and time-consuming. As the demand for new therapeutic increases, efficient, and robust methods to improve the production and screening processes of recombinant proteins in this expression system are necessary to respond to large-scale manufacturing needs. Research has shown that tailored media supplementation and optimization is an efficient, and useful strategy to reach high density cultures and increase protein production in-vitro. Therefore, this project aimed to evaluate the effects of two macromolecules (glucose in high concentration, and glutathione) as potential cell culture additives or boosters to increase the production of therapeutic proteins in the baculovirus/insect cell expression system. It was demonstrated that glutathione addition resulted in a more rapid, universal production of protein compared to minimal media, suggesting this to be a valuable addition to IC-BEVS media.

Protein-based therapeutics are highly potent medicines produced by various expression systems to prevent, treat, and cure a variety of diseases such as cancer, autoimmune diseases, and infectious diseases, as well as genetic disorders. Several currently approved human therapeutics are manufactured using IC-BEVS due to its easy adaptation to serum-free media, high levels of protein expression and post-translational modifications, and its scalability for manufacturing.[1,2] Recombinant proteins produced in this system may constitute antibodies, vaccines, growth factors, hormones, interleukins and pro-inflammatory cytokines.[3] Currently, recombinant proteins are most often produced in expression systems such as yeast, bacteria, insect cells, mammalian cells, and plant cells.[4]

Insect cell/baculovirus expression system (IC-BEVS) are well-established in the pharmaceutical and biotechnological industries to produce recombinant proteins. This expression system is particularly used due to the natural propensity of baculoviruses to infect insect cells.[2] In manufacturing, the most commonly used baculovirus is the Autographa Californica multiple-capsid nuclear polyhedrosis virus (AcMNPV) which is a double-stranded DNA virus with a genome of approximately 135 kb, which is capable of infecting multiple insect cells such as the Spodoptera frugiperda,from which the Sf9 cell line is derived.[5] Because of its large size, BEVS can accommodate larger inserts consisting of single or multiple genes; making it an ideal system to produce complex recombinant proteins.[1] Since its development in 1983, this platform has been effectively used to produce human and veterinary therapeutics.[6,7] Although scientists have developed cutting-edge tools to produce these therapeutics, there remains a great need to improve production efficiency while minimizing cost.

One major drawback of IC-BEVS is that baculovirus infection induces cellular apoptosis and lysis in insect cells, which reduces the longevity of the culture and impedes reinfection of these cells.[8,9,10] This early cell death may be due to the accumulation of reactive oxygen species (ROS) in the mitochondria of these cells upon viral infection.[8] Therefore, protein production in this platform is restricted to about three days post-infection. To remedy this issue, researchers have developed multiple strategies to increase the longevity of SF9 cultures post infection. One of the most used strategies is the use of highly specialized cell culture media. Previous research has demonstrated that the optimization of cell culture parameters, media components, and feed strategies aid in obtaining high-density cultures which result in higher yields of recombinant proteins in the baculovirus/insect cell expression system.[5]

Certain additivess such as glucose, cholesterol, and antioxidants have been shown to improve production yields and quality of recombinant proteins in the IC-BEVS system.[10] Glucose supplementation, in particular, as the main source of energy for uninfected SF9 cells, increases the longevity and supports higher density SF9 cell cultures.[11,12] Moreover, although poorly

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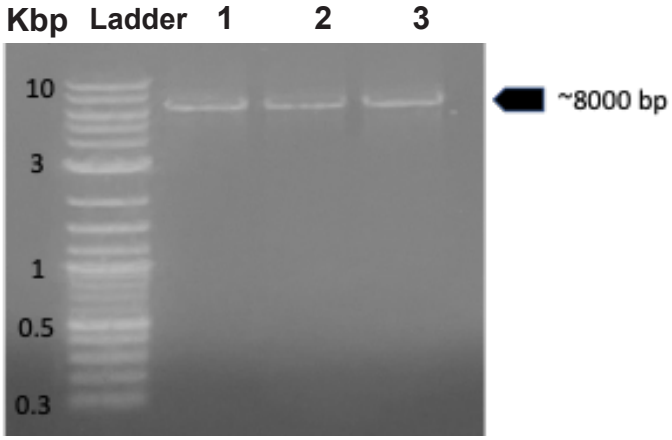

Figure 1 | The KIF1C gene is present in the newly made recombinant plasmid.
Agarose gel electrophoresis of KIF1C PCR fragment amplified from the recombinant baculovirus using flanking m13 forward and reverse primers. Electrophoresis was performed for 1 hour at 100 volts. A 1kb plus DNA ladder was used. Lanes 1, 2, and 3 contained three distinct colonies picked from a transformation plate.

studied in insect cells, the antioxidant, glutathione, acts as a Radical Oxygen Species scavenger and protects mammalian cells from premature cell death.[13] More recent studies by Monteiro et al., extend the idea that glutathione is a promising addition to insect cell culture media as well.[10]

Therefore, this research project aimed to examine the effects of the two macromolecules, glucose and glutathione, as cell culture media additives to increase the yield of recombinant proteins in the IC-BEVS system. Protein expression and production were analyzed using flow cytometry and spectrophotometry.

Materials and Methods

Cell culture and maintenance

The *Spodoptera frugiperda*-derived Sf9 cell line (ThermoFisher, Cat# 11496015) was maintained in Sf-900TM II (ThermoFisher, Cat #10902088) serum-free, protein-free insect cell culture medium in 125 mL Erlenmeyer flasks with 30 mL working volume. Cultures were kept in a 27ºC, non-humidified incubator, oscillating at 130 rpm. Cultures were split routinely, to maintain a density of 3-5x10^6 cells/mL, every 3-4 days. Cell concentration and viability were determined and evaluated by the hemocytometer and trypan blue exclusion method.

Baculoviruses and stock of recombinant bacmids were prepared using the Bac-to-Bac™ Baculovirus Expression System (ThermoFisher, Cat# 10359016). A pFastBac plasmid encoding for the KIF1C kinesin family member, fused N-terminally with a poly-histidine tag and C-terminally with GFP, pFastBac-M13-6His-KIF1C-GFP, was a gift from Anne Straube (Addgene plasmid # 130975 ; http://n2t.net/addgene:130975 ; RRID:Addgene_130975). Gene insertion was accomplished by co-transfection with these vectors and monitored using X-Gal blue-white screening. This was confirmed by PCR using recombinant bacmid as template and pUC/M13 forward and reverse primers. PCR product was run on a 0.8% agarose gel. P0 virus stock was generated by transfecting 25 mL of Sf9 suspension cells in Sf-f900TM (viability was greater than 95% and density was at 2.5x10^6 cells/mL) in a 125 ml shake culture flask. For each transfection sample, a mix of 30 µL of ExpiFectamineTM Sf Transfection Reagent (ThermoFisher, Cat# A38915), 1 mL Opti-MEMTM I Reduced Serum Medium (ThermoFisher, Cat#31985070) and 12.5 µg of undiluted recombinant bacmid was added dropwise onto the cells. Cells were incubated at 27º C for 72-96 hours in non-humidified, non-CO_2 incubator while oscillating at 130 rpm or until visible signs of virus infection. Upon reaching 50% viability, culture was harvested and centrifuged at 1700g for 10 min. The supernatant was stored at 4°C and protected from light to be used for virus titration and/or as inoculum in subsequent infections.

Baculovirus titration and total particles quantification

Virus titers were to be determined by a plaque assay. Briefly, a 6-well plate was seeded with 0.5×10^5 cells/mL per well, where serial dilutions of the viral sample was inoculated. Two replicates for each dilution were performed on the same plate Sf-f900TM insect culture media was used as negative control. Plates was screened after 7 to 10 days for plaque formation. The relative number of viral particles (titer) was to be calculated using the following equation: Titer (pfu)/mL = number of Plaques / (Dilution factor x Volume of inoculum per well).

Additives preparation

A stock of 100 g/L of glucose (Thermofisher, Cat# A16828), and a stock of 50mM of Glutathione (SigmaAldrich, Cat#G4251) were prepared in deionized water, filter sterilized, and stored according to the manufacturer's instructions. Each stock was diluted accordingly to test cytotoxicity on Sf9 cells.

Cytotoxicity of additives on SF9 cells

To determine the optimal concentration of each additive, SF9 cells were seeded in a 12-well plates, treated with different concentrations of glucose or glutathione, and incubated in a non-humidified, non-CO_2 incubator at 27ºC for three days. Samples were then collected and diluted using a dilution scheme of 10x using Sf-900TM II (ThermoFisher, Cat #10902088) serum-free, protein-free insect cell culture medium for a total volume of 1 mL of cell suspension. After dilution, each sample was treated with SYTOX AADvanced Ready Flow (Invitrogen, Cat. No. R37173) as described on ThermoFisher.com.[14] Samples were then analyzed using the Attune NxT Flow Cytometer. The relative cell density, as well as the percentage of live and dead cells were determined.

Cell growth, infection, and production of recombinant protein

Sf9 cells (ThermoFisher) were seeded with the optimal additive concentration (determined in previous Sytox viability/cytotoxicity assay) and infected at 1.0x10^6 cells/mL with an MOI of 5 pfu/cell with baculovirus-expressing GFP, using 125-mL non-baffled, vented shake flasks The samples were incubated in a non-humidified, non-CO_2 incubator at 120 rpm and 27ºC.

This study included control shake flasks also incubated at 27ºC with agitation at 120 rpm: Sf9 seeded at $1.0x10^6$ in a non-supplemented medium, Sf9 cells seeded at $1.0x10^6$ in supplemented medium, and Sf9 cells seeded and infected at $1.0x10^6$ in a non-supplemented medium with a multiplicity of infection (MOI) of 5 pfu/cell. Samples was collected daily for five consecutive days to assess protein production and viability of cells post-infection.

Viability of cells post-infection

Viability of Sf9 infected cells was determined by SYTOX™ AADvanced™ Dead Cell Stain (ThermoFisher, Cat# S10274) assay as described on ThermoFisher.com14 every day for five consecutive days. Each sample was then diluted using a dilution scheme of 10x using Sf-900TM II (ThermoFisher, Cat #10902088) serum-free, protein-free insect cell culture medium for a total volume of 1 mL of cell suspension. After dilution, each sample was treated with SYTOX AADvanced Ready Flow (Invitrogen, Cat. No. R37173) as described on ThermoFisher.com.[14] Samples were then analyzed using the Attune NxT Flow Cytometer.

Relative Quantification of Recombinant Protein

GFP fluorescence was measured two ways: GFP expression inside the cells via flow cytometry, and GFP release in the culture environment via spectrophotometry.

To detect GFP expression inside the cells, samples of cell suspension were collected and diluted using a dilution scheme of 10x using Sf-900TM II (ThermoFisher, Cat #10902088) serum-free, protein-free insect cell culture medium for a total volume of 1 mL of cell suspension. After dilution, samples were analyzed using the Attune NxT Flow Cytometer.

To detect GFP release in the cell culture media, samples of cell suspension were collected and centrifuged at 500g for 5 minutes. Supernatant were then collected and analyzed on a SpectraMax M3 using an excitation max of 488 nm and an emission max of 510 nm.

Results

KIF1C gene insertion into baculovirus construct was confirmed via PCR and gel electrophoresis.

In order to produce a baculovirus construct that can successfully infect insect cells to produce a protein, DH10Bac E.coli cells -containing bacmid construct-were chemically transformed with pfastbac vector encoding for a kinesin-like protein c-terminally linked to a GFP gene and n-terminally linked to a poly-histidine tag. The presence of the mini-attTn7 attachment site on the bacmid constructs allows proper transposition of the KIF1C genes into the bacmid construct which disrupts the expression of the LacZ peptide. When the newly transformed cells are grown on a plate, the colonies that express the LacZ gene due to the absence of the KIF1C gene appeared blue while the colonies expressing the KIF1C gene due to disruption of the LacZ gene, appeared white. Three white colonies and three blue colonies were picked from plates, then grown in liquid broth.

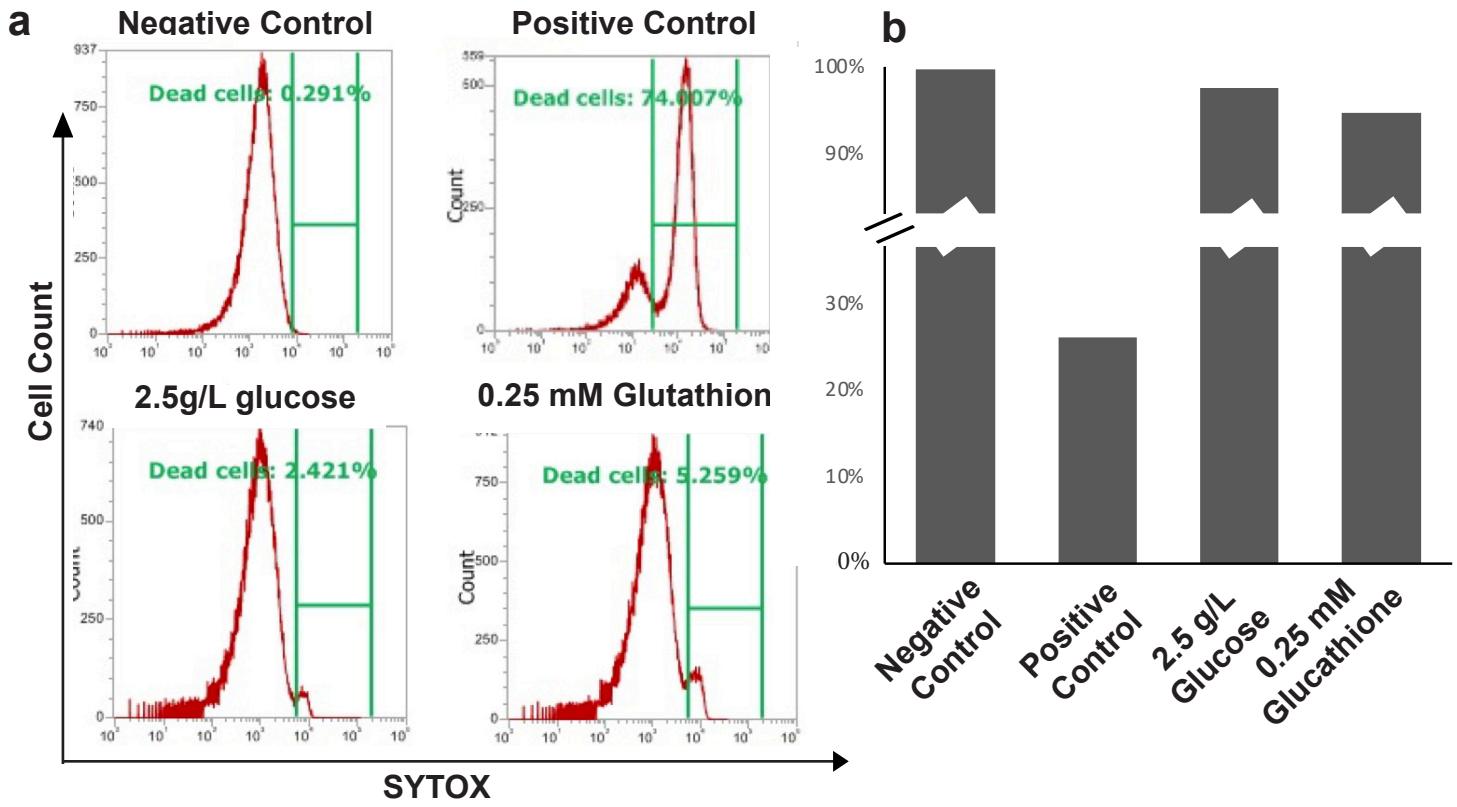

Figure 2 | Glucose and Glutathione Media Supplements maintained cell viability.
Cells were grown at 27º C for three days, agitated at 125rpm, and with a variety of concentrations of glucose or glutathione. Following incubation, cytotoxicity and cell viability were assessed using SYTOX AADvanced Ready Flow on an Attune NxT Flow Cytometer. Negative control represents a culture grown in basal (unsupplemented) media. Positive control represents a culture grown in basal media treated with 10% ethanol for three days. (**a**) Histograms of SYTOX AADvanced incubation with or without additives. (**b**) Bar graph of percent viability with basal media vs basal media + additives (as indicated).

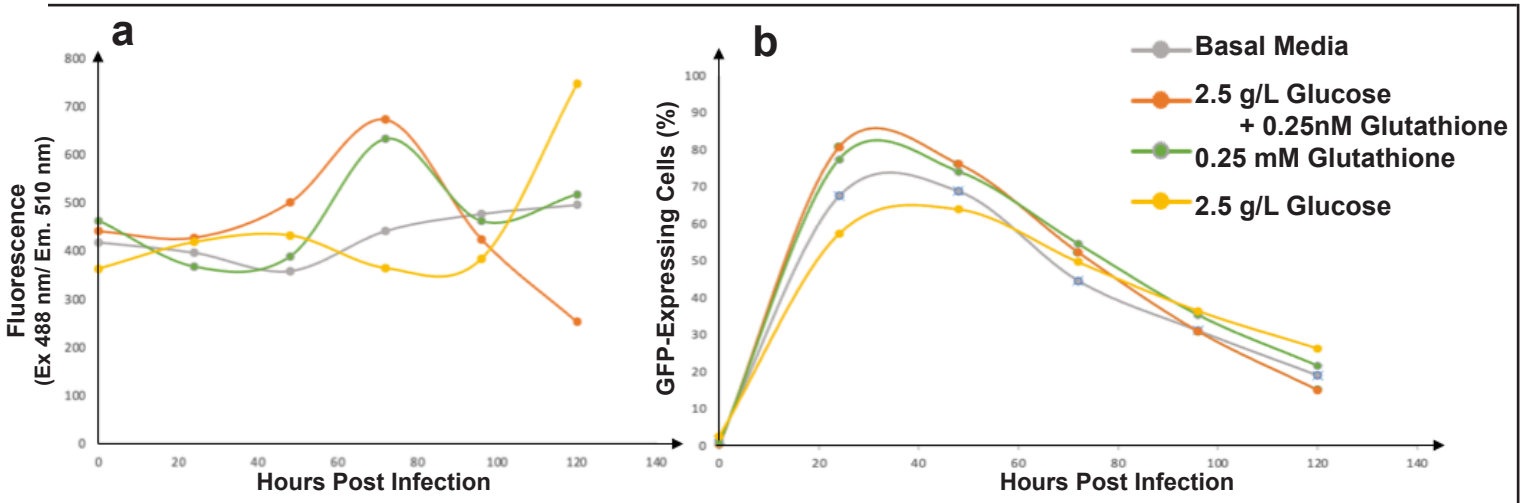

Figure 3 | Relative Quantitation of soluble, secreted- and cellular- GFP.
Sf9 cells were seeded at 1 x 10^6 cells/mL and infected at MOI 5 pfu/mL. Samples from each culture were collected daily over five days. **(a)** Relative secreted GFP fluorescence intensity was measured in spent media at the indicated times post-infection. **(b)** Relative cellular GFP fluorescence was analyzed by flow cytometry.

KF1C-expressing baculovirus were then extracted and subjected to a PCR using m13 forward and reverse primers to confirm proper transposition. A gel electrophoresis of the PCR product was performed. As expected, a single band of 8000 base pairs was seen on the gel, indicating the presence of the KIF1C gene in the recombinant bacmid (**Figure 1**).

Titration of baculovirus expressing KF1C

A plaque assay was performed to determine the viral titer of the baculovirus-expressing KIF1C stock. Contrary to what was expected, after 7 days of incubation, no plaque formation was observed. This assay was repeated twice over, with the same results obtained: No plaque formation. It was hypothesized that the method used to transfect Sf9 cells in the previous step was faulty, accounting for the lack of baculovirus produced. Due to lack of time, a baculovirus-expressing GFP was used for the remaining of this project to test if the addition of glucose and glutathione to basal media affects the production of protein the IC-BEVS system.

Addition of glucose and glutathione helped maintain high viability of uninfected cells.

Before assessing protein production, the effects of glucose and/or glutathione on cell viability were assessed using flow cytometry. SF9 cells were incubated with different concentrations of glucose (starting from 1 g/L to 30 g/L) and glutathione (starting from 0.25 mM to 15 mM), or with basal media for three days. Cells from each condition were harvested and treated with SYTOX dye. The percentage of dead cells were determined by the uptake by the SYTOX dye by dead cells. The negative control, which represents cells grown in basal media, maintained a viability of 99.5%. Cells treated with 2.5 g/L glucose only maintained the highest viability (97.6%) of all the different concentrations tested. Among the different concentration tested, cells treated with 0.25 mM of glutathione maintained a viability of 94.7% (**Figure 2b** and data not shown). These concentrations were used moving forward in this project.

Relative quantitation of GFP post-infection

To determine the effect of glucose and glutathione on production of GFP after infection with a baculovirus, the relative production of GFP produced after infection with the baculovirus stock was measured two ways: GFP expression inside the infected SF9 cells via flow cytometry, and media analysis of GFP released in the culture post-infection via spectrophotometry. Cells were seeded with optimal concentration of additive determined in prior viability assay then infected with baculovirus expressing GFP with a MOI of 5pfu/mL. **Figure 3a** illustrates the relative intensity of GFP released in the cell culture environment post infection. Glutathione seemed to favor rapid production and release of GFP. A maximum concentration seemed to occur at 72-hour post infection. Glucose on the other end seems to favor GFP release much later in the infection which occurred around day 5 post-infection, while basal media showed a steady release of secreted GFP in the media. **Figure 3b** illustrates the relative fluorescence intensity inside Sf9 cells post infection. Interestingly, the cells demonstrated a similar pattern of GFP expression, no matter the conditions they were grown and infected in. Expression of GFP seemed to peak between 24 to 48 hours post infection and then tailed off thereafter. Again, cells grown in glutathione-supplemented media seemed to favor higher expression of GFP.

Viability post-infection

To elucidate the possible cause of the early release of GFP in the media when the expressing cells were grown in glutathione, the viability of these Sf9 cells post-infection was analyzed using the SYTOX AAdvanced reagent on cells three or five days post-infection using the flow cytometer. As baculovirus is a lytic virus, we hypothesized that, contrary to what was seen in the prior experiments using uninfected cells, glutathione might not impede cell death in infected cells, resulting in the rapid release of GFP. As the highest level of expression and release of GFP was observed during day 3 post-infection and day 5 post-infection, the viability of the infected Sf9 cells was analyzed during these timepoints (**Figure 4**).

Already, at day 3 post-infection, the cells grown in glucose-supplemented media and basal media were less viable compared to the one infected in a glutathione. However, by day 5 post-infection, no matter the conditions, the cells appeared to be dead, similar to the positive control.

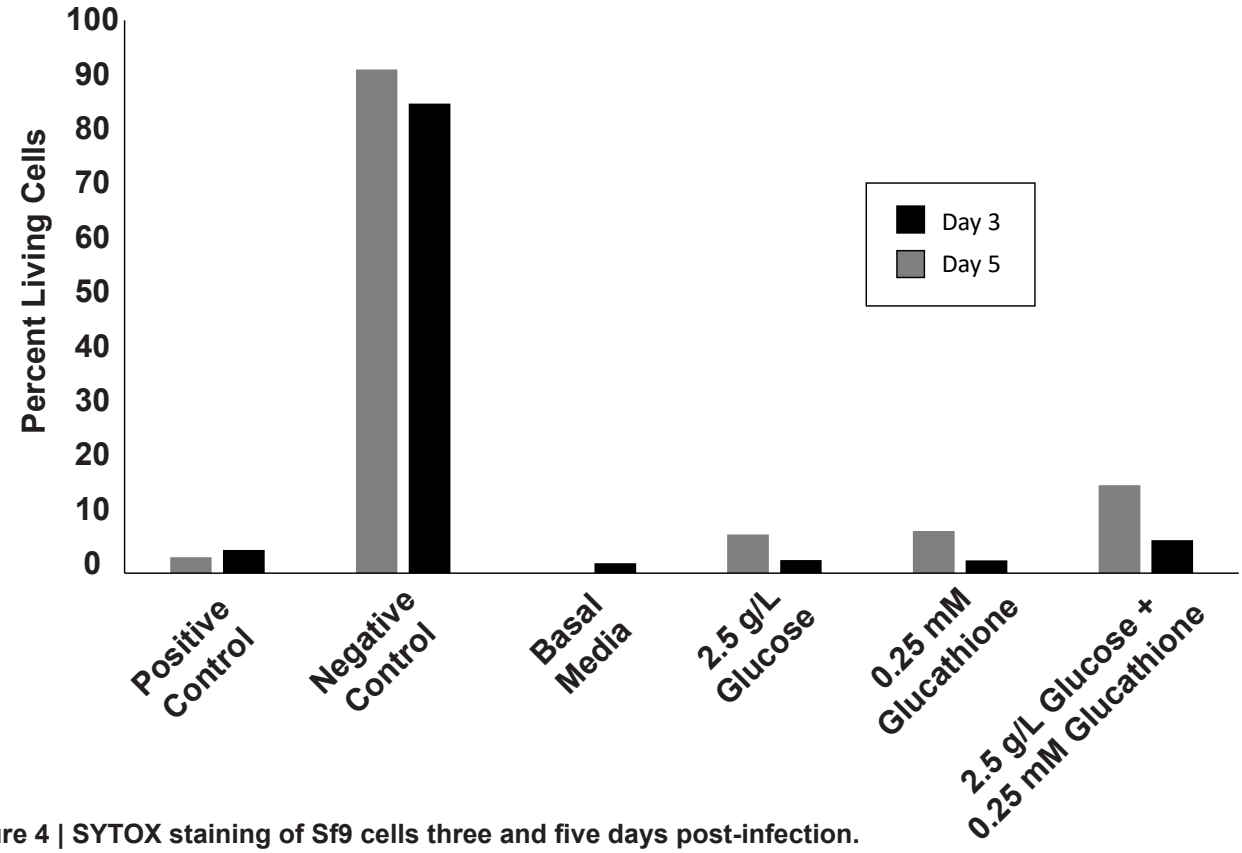

Figure 4 | SYTOX staining of Sf9 cells three and five days post-infection.
Cells were infected and grown at 27º C for three days, agitated at 125rpm, and with basal media, or basal media supplemented with 2.5 g/L glucose, 2.5mM glutathione, or both. The positive control consisted of uninfected sf9 cells grown in basal media, then treated for 10% ethanol for three days. The negative control represents a culture of non-infected sf9 cells grown in basal media. Basal media represents infected cells grown in media without supplementation. Graphs depict the percentage of SYTOX AADvanced-unstained (living) cells from the various conditions indicated at either 3 or 5 days post infection.

Discussion

The assays run during this project did not paint a full picture. The did not elucidate the reason why there was an early release of GFP in a glutathione supplemented media, and a late release in a glucose supplemented media. But nonetheless, from the data gathered, the authors observed that both glutathione and glucose seemed to aid in maintaining high viability in uninfected Sf9 cell cultures. Glutathione might also aid in the early and rapid release of recombinant proteins in-vitro as well as maintaining higher viability post-infection. However more research is required to better understand mechanism of infection, as well as the correlation between nutrients availability and protein production in the baculovirus/insect cells expression system.

Author Biography

Guenaele Raphael is a senior in Biotechnology at the University of Kansas. After graduation, she hopes to continue working in the biotechnology industry where she can gain experience in research and development of new therapeutics.

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Author Contributions

G.A.R contributed to the experimental work, design, and writing of this work; J.F.T. contributed to the editing and design of this work; R.L.L. contributed to the editing and design of this work.

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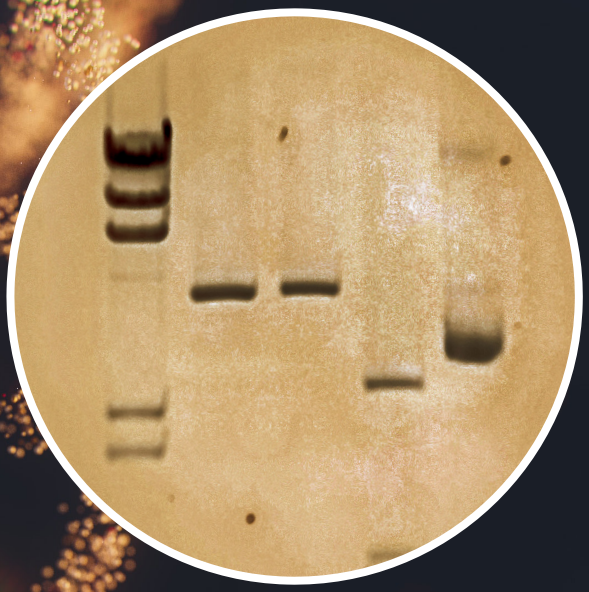

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