MOLECULAR INTERACTIONS OF HUMAN CELL PROTEINS WITH SARS-COV-2 VERSUS INFLUENZA VIRUSES

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MOLECULAR INTERACTIONS OF HUMAN CELL PROTEINS WITH SARS-COV-2
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by

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ABSTRACT

Molecular Interactions of Human Cell Proteins with SARS-CoV-2 versus Influenza Viruses

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The novel Coronavirus Disease 2019, or COVID-19, shows considerable similarity to a common influenza infection. Over the past year, innumerable studies have been investigating the virus in various capacities, from big-picture public health repercussions to the virus’ biochemical interactions with human cells for infection. This research provides invaluable information in the ongoing war against infectious diseases. While the influenza virus is fairly well-understood, much surrounding the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains unclear, including its longevity, long-term health effects, methods of transmissibility, and more. Both the scientific community and the general public are asking the same question: how can the two appear so similar yet be so different? We must probe their differences at the most fundamental level before such inquiries can be properly addressed. The purpose of this article is to evaluate and compare the molecular interactions of each virus with human host cells and draw inferences about anomalous qualities observed through SARS-CoV-2 infections. Examining the genomic characteristics of antibodies created to combat each
virus – both those from a naturally occurring infection and vaccine-induced – may provide insight into infection, immunity, and potential cross-reactions. COVID-19 has catapulted the world’s population through a total societal upheaval with economic, intrapersonal, and political ramifications, the likes of which have not been seen in a century – since the 1918 H1N1 influenza virus outbreak. Each pandemic event exposes the weaknesses of the healthcare system and better prepares us for the next. The conclusion of this article will hypothesize what a new “normal” may look like and which avenues of medicinal and technological advancement may help us avoid or better prepare for the next global pandemic.

KEYWORDS: Molecular Structure, Viral Infection, Replication, Microbiology, Immunology
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CHAPTER ONE

Introduction

This article is a literature review that will broadly cover topics relating to the influenza virus and SARS-CoV-2/COVID-19, primarily focusing on their genetic composition and molecular structure. It will explore, in-depth, the protein interactions of each virion with healthy human cells and antibodies. Through comparison research methods, I plan to identify some, as of yet, unexplained anomalies observed during SARS-CoV-2 infections and formulate hypotheses regarding their causes and potential long-term effects. There is a lack of research concerning the long-term effects of COVID-19, and this article neither produces nor claims to produce any new, supported evidence concerning this topic. This shortage leaves ample room for the discussion of medicinal and scientific outlook on managing and predicting infectious diseases.

Several primary, peer-reviewed articles from various chemistry and biology journals were reviewed, and relevant information was compiled into this secondary literature format. Many sources covering SARS-CoV-2 take an in-depth look at the molecular structure of the virion and isolate its interactions with human cells to determine its mechanism of infection and replication. These observations will lead to a brief discussion about potential antiviral targeting and vaccinations.
CHAPTER TWO

Molecular and Genomic Foundations of Influenza

Physical Structure and Function

The influenza virus originates from the *Orthomyxoviridae* family which are characterized by their negative-sense, single-stranded RNA genomes (Bouvier and Palese, 2008). They are further classified as type A, B, or C based on the ratio of structural to nonstructural proteins. For the purposes of this literature review, we will only discuss types A and B and further focus solely on strain A. Both type A and B are comprised of eight, negative-sense, single-stranded viral RNA segments (Bouvier and Palese, 2008). Influenza A RNA segments encode nine structural and two nonstructural proteins whereas influenza B RNA encodes 10 structural and one nonstructural protein (Lofgren et al., 2007). By electron microscopy, influenza A and B viruses are virtually indistinguishable with a conserved spherical or filamentous shape of similar diameter and length (Bouvier and Palese, 2008). Their differences are apparent at an atomic level through meticulous examination of their integral membrane proteins. Strain A has three, namely hemagglutinin (HA), neuraminidase (NA), and matrix-2 M2 (Figure 1); strain B has four (HA, NA, NB, BM2), but they serve the same basic function of

![Influenza A virus](image)
enclosing the virion core which consists of a nuclear export protein (NEP) and ribonucleoprotein (RNP) (Bouvier and Palese, 2008). HA and NA serve to bind and release the virion from the host cell, respectively. M2 and BM2 are strain-specific — indicated by the absence (A) or presence (B/C) of a letter before M2 — proton conductors which equilibrate pH within the virion. The functional necessity of NB is not currently accepted, but it is thought to serve as an ion channel (Hatta & Kawaoka, 2003). The internal nonstructural proteins comprise the viral RNA segments and the co-requisite RNA-dependent RNA polymerase — a necessary tool for the virus to replicate after entry and genome integration within the host cell. The minor structural differences between influenza A and B play a larger role in their infectivity, symptom presentation, and virulence.

**Infection and Disease**

These viruses are the primary cause of an acute viral respiratory disease known as the flu. Influenza infection leads to the hospitalization of over 200,000 people yearly and results in 36,000 deaths from flu or flu-related complications in the United States (Lofgren et al., 2007). It affects the whole body with symptoms such as fatigue, high fever, and muscle aches, but a majority of the hallmark symptoms occur throughout the respiratory tract, including cough, runny or stuffy nose, shortness of breath, and a sore throat, all of which onset one to four days after initial exposure. The region of infection concentration in the upper respiratory system (bronchi, nasopharynx, sinus cavities, and trachea) lends to its highly contagious nature as a direct result of the increased susceptibility of alpha-2,6 epithelial cells to airborne virus particles. The lower respiratory tract’s (alveoli and bronchioles) alpha-2,3 cells are more protected, but their
infection often coincides with more serious repercussions. Influenza’s annual recurrence and strong prominence, though limited by its seasonal duration, underlines the gaps in modern understanding of disease evolution and seasonality.

Influenza is categorized as a seasonal virus, meaning the virus and subsequent infection rates are especially prevalent during one time of year; this marked increase typically occurs during the winter months. Though its seasonality is one of the most widely observed phenomena of infectious disease, it remains one of the most poorly understood (Lofgren et al., 2007). Various scientific disciplines have contributed to exploring the underlying causes of this seasonal pattern, but the links and gaps have yet to be connected and filled in, respectively, though assumptions can be drawn from knowledge that the colder weather of the season forces more indoor activities, therefore more contagious events. There are several factors that make influenza so difficult to predict including viral evolution, seasonal host health, and social and environmental causes, such as air travel, ambient temperature, and crowding (Lofgren et al., 2007). The overwhelming nature of flu season is due, in large part, to secondary bacterial infections that occur subsequent to a primary influenza viral infection. Certain age groups are particularly susceptible to this occurrence as a result of pre-existing conditions, including cardiovascular health complications and old age. Secondary infections commonly arise from encapsulated bacteria species *Streptococcus pneumoniae* and *Staphylococcus aureus* infiltrating a host with an overwhelmed innate immune response (Metzger and Sun, 2014). Various cell types are involved in combatting a viral infection as it has a unique mechanism of infection and replication by hijacking host cell machinery.
Infection Mechanism

Influenza virion spikes, which are later discussed in depth, recognize linkages and steric configurations on host cells that are unique to certain cell types and locations. For example, alpha-2,3 binding proteins help restrict species infectivity, but that infection remains possible due to the presence of sialylated proteins deeper in lung tissue, which explains the low infectivity but high pathogenic rates (Bouvier and Palese, 2008). All members of the Orthomyxoviridae family are enveloped RNA viruses which employ a

lytic replication cycle within the host cell cytoplasm. Special viral-encoded proteins are necessary for this mode of replication. The whole infection cycle consists of six steps (Figure 2) beginning with the aforementioned recognition and attachment to sialic acid host cell receptors –

Figure 2 – Influenza virus lifecycle and antibody targets (Krammer, 2019)
alpha-2,3 or alpha-2,6 depending on infection location. After attaching to the cell surface, the virus enters the cell via endocytosis, viropexis, or membrane fusion (strain-dependent mechanism); either before, during, or after entering the cell, the virion must uncoat itself in order to release its genomic contents from the capsid and begin replicating. Negative-sense, single-strand RNA genomes are quite complex as human ribosomes will not recognize and translate this RNA directly. They must encode and pre-assemble an RNA-dependent RNA polymerase, which is attached with the genome in the capsid and makes complementary positive-sense messenger RNA (mRNA) from the negative strand template. The complementary mRNA is used to make protein and serves as the template to produce more negative-sense, single-strand RNA from which the cycle repeats. Thermodynamic assembly and maturation of new virions occurs with the help of matrix proteins that direct nucleocapsids where to leave the cell with their related surface proteins, primarily HA and NA. Fusion proteins assist their release via cell lysis or budding. This lifecycle is highly conserved across enveloped animal viruses. Recently, one similar virus of particular interest and relevance is SARS-CoV-2.
CHAPTER THREE

Molecular and Genomic Foundations of SARS-CoV-2

Viral Origin and Disease Presentation

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the identified cause of the coronavirus disease 2019 (COVID-19). The first cases were reported in

Wuhan, China

in December 2019, and it has since evolved into a worldwide pandemic outbreak, infecting over 29 million people, and killing more than 530,000 in the United States alone (CDC COVID Data Tracker) as of March 2021.

Though it is currently unknown why, infected persons may present with symptoms ranging from asymptomatic through severe infections that can lead to death. Although symptoms vary based on host health, they are all characterized as acute onset (two to 14
days after exposure) and may include cough, dyspnea, fever, loss of smell and taste, myalgia, and evidence of ground-glass lung opacities (Sanda et al., 2021). Bearing the resemblance to symptoms of and the system afflicted by the flu, one can reasonably infer that SARS-CoV-2 spreads in a fashion similar to influenza – via airborne virion particles – though there was much confusion early on among the scientific community as to whether this was the case. Studies have confirmed this mechanism of transmissibility while also providing evidence that COVID-19 is more contagious and spreads more quickly (He et al., 2020, Petersen et al., 2020). The apparent similarities of coinfection and symptom presentation (Table 1) initially led many to compare the SARS-CoV-2 and influenza viruses. There are several more notable differences between the two, specifically regarding long-term effects and mortality, which will be discussed in-depth in a later section. Although COVID-19 has rightfully received global attention for its novelty and profoundly damaging public health effects, SARS-CoV-2 is not the first of its kind.

Molecular Structure

There are several subspecies encompassed under the umbrella term “coronavirus” – all categorized by their crown-like appearance from the externally protruding spike proteins on their surface (Figure 3). Though SARS-CoV-2 is not the only virion with these structures, the spikes are incredibly important in aiding our understanding of the virus’ host selection and infectivity. They possess a unique flexibility and orientational
freedom (Figure 3) (Kiss et al., 2021), granting them the ability to search the host for a binding receptor. The first step for coronaviruses to invade host cells is binding of the spike receptor-binding domain (RBD) to angiotensin I converting enzyme 2 (ACE2) proteins which are only expressed on certain tissue cell types and work to modulate blood pressure and inflammation. Through biochemical experimentation, SARS-CoV-2 shows evidence of optimization for binding to the human ACE2 receptor (Andersen et al., 2020). Notably, however, ACE2 expression on respiratory tissue is limited at both the mRNA and protein level (Hikmet et al., 2020). This is interesting considering how heavily affected the respiratory system is, though various organ systems express functional consequences following COVID-19 infection, such as cardiovascular and renal complications which have been reported in several studies (Murk et al., 2020). Humans’ have a high susceptibility to inhaled viruses which leads most infectious viruses to have the ability to infect and replicate within airway epithelial cells (Hikmet et al., 2020). Oftentimes, if these comorbidities are pre-existing conditions, there has been an observed increase in the severity of symptoms and mortality rate. Though not entirely unique to SARS-CoV-2 virions, it is a distinguishing characteristic that they are able to infect various cell types from various organ systems.

**Infection and Replication Processes**

To the virion’s advantage, the RBD in the spike protein is the most variable part of the coronavirus genome (Andersen et al., 2020), giving it a highly adaptable nature. This, in conjunction with the positive-sense, single-stranded RNA genome, leads to logical predictions of diverse strain variability because of the error-prone nature of viral RNAs. Positive-sense RNA naturally runs 5' to 3' – the pattern necessary for a viral sequence to
be directly translated to proteins – thus can be immediately translated by the host cell in replication processes; this process is incredibly susceptible to errors because vRNA genomes do not employ error-correcting mechanisms. SARS-CoV-2 must undergo attachment and nucleocapsid uncoating – just as influenza does – because it is an enveloped virus. After successful attachment, cellular proteases must cleave the spike protein at the functional polybasic (furin) cleavage site located at the junction of S1 and S2, the two spike monomer subunits (Andersen et al., 2020) (Figure 4); this action is necessary for fusion of viral and cellular membranes and genome integration to occur (Hikmet et al., 2020). Binding is accomplished via a dynamic search mechanism of a host cell by the virion’s external spike glycoproteins, and interaction with the host ACE2 glycoprotein mediates entry (Sanda et al., 2021). SARS-CoV-2 spikes form a trimeric structure on the surface of the viral envelope, each one consisting of an S1 and S2 subunit (Sanda et al., 2021) which help mediate binding and entry, respectively. Once incorporated, the virion can begin hijacking host cell machinery to produce proteins necessary for its own replication needs; SARS-CoV-2 positive-sense genome acts as mRNA which can be directly translated by host cell machinery to produce these viral proteins. It must encode for an RNA-dependent RNA polymerase when it
arrives inside the host cell because human cells are unable to produce RNA from an RNA template. The polymerase turns out complementary negative-sense, single-stranded RNA, which then serves as a template for more positive-sense, single-stranded RNA from which proteins are produced. This type of genome is usually translated as a single polyprotein that is subsequently cleaved by the host cell or viral proteases. The virions acquire their characteristic spikes during viral assembly and maturation. Notably, the number of spikes on an individual virion is highly variable; this detail may be regulated during production and may be a result of several environmental factors within the host cell (Kiss et al., 2021). Much about SARS-CoV-2 is taking what little it has and making its environment work for itself; this becomes especially apparent when observing its functional and mechanical properties.

These viral particles are incredibly complex and evolved nanostructures. Studies have shown them to be highly compliant and resilient; they have exhibited a remarkably temperature-resistant structure and the ability to fully recover from drastic mechanical perturbations which may lend to their aerosol and surface stabilities (Kiss et al., 2021). The temperature dependence of the virion has not yet been fully explored, but initial studies show partial topography restoration with the spike proteins being the most affected with reductions in both number and size (Kiss et al., 2021). These conformational distortions suggest thermal denaturation through progressive dissociation of the spike trimers, eventually leading to inactivation of the virion (Kiss et al., 2021). Still, there is persistent structural self-healing observed which is likely a result of dynamic interactions between their genomic (RNA), lipid, and protein components (Kiss et al., 2021). There are various unique aspects that characterize SARS-CoV-2 and make it
difficult to understand. These observations breed questions. Influenza currently serves as a valuable comparison tool to aid our knowledge of the novel virus’ infectivity, transmission, virulence, etc.
CHAPTER FOUR

Likenesses and Variances

As has been noted at several junctures, SARS-CoV-2 shares many similarities with influenza, including symptom presentation, basic cell structure (enveloped, single-stranded RNA genome), and a lytic replication cycle. Ironically, in the same ways that they are alike, they are still quite different from one another: symptoms appear at different times and last for different durations, SARS-CoV-2 infection may display a characteristic loss of taste and smell not observed through any other infection, and the SARS-CoV-2 genome is positive-sense and non-segmented whereas influenza’s is negative-sense and has multiple segments. Due to the recency of these observed phenomena, there are hypotheses to be formulated from these undefined observations. For what seemed like the first time in a century, scientists and the general population alike were taken aback and asking the same questions about a novel disease. What causes the severity level of an infection? Why do most people show more severe symptoms when infected by SARS-CoV-2 than by influenza virus? How do their strikingly similar molecular interactions still evoke such different responses from human cells? While it seems that there is an acute shortage of answers, some conclusions can be inferred from what has been observed and researched thus far.
Transmission

One of the more controversial aspects of the 2020 pandemic — notably, most of the controversy was concentrated in the United States — was the government mandate to wear face masks that cover one’s mouth and nose in an effort to slow the spread of the virus.

Despite what anyone’s personal opinion on the matter may be, there is indisputable evidence in a graph from the South Dakota Department of Health that wearing a face covering helps reduce the spread of airborne viral particles. The chart (Figure 5) displays an extreme reduction in influenza rates during the 2020-2021 season after four years of continually magnifying increases. These unusually low numbers are also likely due, in part, to social distancing efforts which would reduce the efficacy of the spread of respiratory droplets. This conclusion is valuable knowledge for combatting the spread of future infectious diseases. Not all revelations on the relationships between the two viruses have been on this macroscopic scale; in fact, there seems to be more answers at the molecular level.
Molecular Interactions

The basic molecular structure of the two viruses is quite comparable; both contain a ribonucleoprotein genome in a protein capsid coat that is enclosed in a lipid envelope with proteins on the external surface (Figure 6). Their compositional similarity lends to the analogous mechanisms of infection employed by each. It is only through scrutinizing the details of their actions, functions, and structures on a fundamental level that their differences, both macro- and microscopic, begin to appear more obvious and have meaning. One disparity relating to both their infectivity and structure is the spikes along the outside of each virion’s envelope. SARS-CoV-2 spike proteins are highly dynamic whereas influenza glycoproteins, both hemagglutinin (HA) and neuraminidase (NA), remain relatively motionless before host cell attachment. The observed rapid spike motion is theorized to contribute to a powerful and effective search of the target cell’s surface (Kiss et al., 2021) for the critical ACE2 binding residues and furin cleavage site (Huang et al., 2020). Individual spike protrusions in SARS-CoV-2 are homotrimeric structures; each monomer is composed of an S1 and S2 subunit connected at the polybasic junction (Verkhivker & Di Paola, 2021). Every S1 domain contains an RBD for the virion, meaning each spike has three binding sites, thereby tripling its binding efficacy (Tang et al., 2021). S2 subunits mediate membrane fusion after cell adherence.
(Tang et al., 2021). Their dynamic nature may help explain why SARS-CoV-2 is seemingly more infectious than influenza, despite having fewer spike proteins (up to 60 observed on SARS-CoV-2 compared to 350 observed on influenza A) (Kiss et al., 2021). This advanced infection mechanism may also contribute to the disparities observed between their symptoms and long-term health effects.

<table>
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<th>Factor</th>
<th>Coronavirus</th>
<th>Influenza</th>
<th>P Value</th>
</tr>
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<tr>
<td>0</td>
<td>1755</td>
<td>3003</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis, y, median (IQR)</td>
<td>61.5 (44.5–73.5)</td>
<td>62.6 (46.5–78.2)</td>
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</tr>
<tr>
<td>Age ≥65 y</td>
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<td>1332 (48.9)</td>
<td>.11</td>
</tr>
<tr>
<td>Diagnosis quarter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1159 (72.0)</td>
<td>2901 (74.5)</td>
<td>&lt;.001</td>
</tr>
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<td>2</td>
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<td>48 (12.1)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>24 (1.3)</td>
<td>10 (0.6)</td>
<td></td>
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<tr>
<td>4</td>
<td>325 (20.5)</td>
<td>501 (12.8)</td>
<td></td>
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<td>Male</td>
<td>641 (41.3)</td>
<td>1359 (43.1)</td>
<td>.21</td>
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<td>3534 (65.6)</td>
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<td>CCC service</td>
<td>112 (7.2)</td>
<td>242 (6.2)</td>
<td>.12</td>
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<td>Pneumonia</td>
<td>254 (15.0)</td>
<td>29 (7.4)</td>
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<td>TID</td>
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<td>629 (15.8)</td>
<td>.40</td>
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<tr>
<td>Death within 30 days</td>
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<td>118 (9.0)</td>
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<td>Coronavirus ICD-10</td>
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</tr>
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<td>218 (54.0)</td>
<td>&lt;.001</td>
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<td>369 (8.6)</td>
<td>.035</td>
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<td>Obesity-TID</td>
<td>144 (9.5)</td>
<td>401 (10.5)</td>
<td>.011</td>
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<td>Obesity-HTN-TID</td>
<td>33 (2.2)</td>
<td>134 (3.3)</td>
<td>.035</td>
</tr>
</tbody>
</table>

Table 2 – Comorbidty factor comparison between patients infected with Coronavirus and those with Influenza (Li, D. et al., 2020)

Abbreviations: ARDS (acute respiratory distress syndrome); CHD (chronic ischemic heart disease); CKD (chronic kidney disease); CLD (chronic lung disease); HTN (essential hypertension); ICD (International Classification of Diseases); T2D (type 2 diabetes)

Comorbidities and Health Outcomes

Compiling evidence continues to support the notion that SARS-CoV-2 has more of a “long-lasting effect” than influenza does. This may be related to the types of comorbidities observed with each infection. Both patients with diagnoses of SARS-CoV-2 and influenza show significant rates of comorbidities, including chronic ischemic heart disease, type 2 diabetes, obesity, and obesity with type 2 diabetes (Table 2) (Li, D. et al., 2020). However, higher rates of chronic kidney disease and chronic lung disease were observed in those with coronavirus infection, whereas influenza patients showed higher rates of diabetes, hypertension, and obesity (Li,
D. et al., 2020). One can appreciate the severity discrepancy between the conditions in that significantly more Americans live with and manage hypertension and obesity than those with systemic organ diseases. Secondary bacterial superinfections commonly follow primary viral infections; adding a third factor to an already overwhelmed defense system may explain why SARS-CoV-2 infections are associated with higher risk of death and infectious pneumonia than influenza (Figure 7), though influenza’s association with bacterial infections is more well-understood (Li, D. et al., 2020).

Granted, death has primarily been observed in at-risk elderly patients with various pre-existing conditions, such as hypertension, coronary heart disease, and diabetes (Li, P. et al., 2021). The SARS-CoV-2 virion’s fundamental interactions and persistence within the body may be a contributing factor to this unfortunate trend. SARS-CoV-2 is the most compliant virus that has ever been investigated with a more malleable lipid envelope than

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**Figure 7 – multivariate analysis of potential health outcomes (Li, D. et al., 2020)**

Abbreviations: CHD (chronic ischemic heart disease); CKD (chronic kidney disease); CLD (chronic lung disease); T2D (type 2 diabetes); HTN (essential hypertension); ICU (intensive care unit)
influenza, lending to increased elasticity, thus the ability to survive mechanically stressful situations (Kiss et al., 2021) and adapt to inhabit different organ systems. While both COVID-19 and the flu are primarily considered respiratory diseases, both causal viruses have been shown to effectively infect and replicate in the intestinal epithelium, causing gastrointestinal symptoms (diarrhea, nausea/vomiting) in patients (Li, P. et al., 2021). This unique ability makes them harder to combat in a clinical setting.
CHAPTER FIVE

Molecular Level Functions and Processes

Alphainfluenzavirus (influenza A) and Betainfluenzavirus (influenza B) are the two most prevalent genera of the Orthomyxoviridae family that infect human hosts. Comprehensively, there are four strains with various subtypes based on surface proteins. The efficacy of antibodies and pharmaceuticals comes from targeting these specific surface proteins. Cleavage or alterations of the chemical or physical structure of the binding proteins essentially deactivates the virion; if it cannot bind, it cannot replicate, leaving it to die via apoptosis or be engulfed by phagocytic immune cells. Most of this article has and will continue to focus on comparisons of influenza A with SARS-CoV-2. Immune responses to any virus are based on previously acquired immunity from prior infections of viral variants. Conserved genomic sequences and structural fragments may trigger immune recognition, initiating a stronger response cascade.

Antigenic Shift

H1N1, a subtype of influenza A (Figure 8), was the causal agent of the 1918 influenza pandemic, and is likely a product of antigenic shift. The unique nature of the influenza virus, specifically its segmented genome and protein function, allow it to employ antigenic drift and

Figure 8 – Ribbon diagram of uncleaved HA monomer from 1918 H1N1 pandemic (Bouvier and Palese, 2008)
antigenic shift – processes that result in viral evasion of long-term adaptive immune responses in human hosts (Bouvier and Palese, 2008). Antigenic shift is the reassortment of influenza RNA segments derived from different viruses and is possible because of influenza’s ability to infect various species. Antigenic drift is the accumulation of a series of minor genetic mutations, eventually producing differentiated antigens, thereby eliciting different antibodies from the host. All influenza A virions are peppered with hemagglutinin (HA) and neuraminidase (NA) glycoproteins projecting from the host cell-derived lipid membrane in a variable ratio of approximately four to one (Bouvier and Palese, 2008). HA’s are responsible for host cell entry, whereas NA’s are responsible for host cell exit (Hagen, 2020). Two of the eight genomic segments inside the virion core encode for the particular surface glycoproteins that will be expressed (Table 3). The remaining segments encode proteins responsible for various functions to ensure the success and viability of the virus in a host (Table 3). Due to the segmented nature of influenza viral RNA (vRNA), the subtypes are susceptible to reassortment, resulting in continual antigenic shift. By one

<table>
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<th>Segment</th>
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<th>Protein length in amino acids</th>
<th>Protein function</th>
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<td>1</td>
<td>2341</td>
<td>PB2</td>
<td>759</td>
<td>Polymerase subunit; mRNA cap recognition</td>
</tr>
<tr>
<td>2</td>
<td>2341</td>
<td>PB1</td>
<td>757</td>
<td>Polymerase subunit; RNA elongation, endonuclease activity</td>
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<td>3</td>
<td>2233</td>
<td>PA</td>
<td>87</td>
<td>Pro-apoptotic activity</td>
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<td>4</td>
<td>1776</td>
<td>HA</td>
<td>550</td>
<td>Surface glycoprotein; major antigen, receptor binding and fusion activities</td>
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<td>1565</td>
<td>NP</td>
<td>498</td>
<td>RNA binding protein; nuclear import regulation</td>
</tr>
<tr>
<td>6</td>
<td>1413</td>
<td>NA</td>
<td>454</td>
<td>Surface glycoprotein; nuclease activity, virus release</td>
</tr>
<tr>
<td>7</td>
<td>1027</td>
<td>M1</td>
<td>252</td>
<td>Matrix protein; vRNP interaction, RNA nuclear export regulation, viral budding</td>
</tr>
<tr>
<td>8</td>
<td>800</td>
<td>NS1</td>
<td>230</td>
<td>Interferon antagonist protein; regulation of host gene expression</td>
</tr>
</tbody>
</table>

Table 3 – Genomic segments of H1N1 virus, their encoded proteins, and protein functions (Bouvier and Palese, 2008)
strain acquiring the HA or NA segment of another subtype, the viral proteins are altered to a novel state that humans lack preexisting immunity against (Bouvier and Palese, 2008). Only three HA and two NA subtypes have caused human epidemics (Bouvier and Palese, 2008), but a new variant within each subtype is observed almost every year. Scientists are continually working to predict how a strain might mutate in an effort to mitigate its impact by producing an effective vaccination. Although the fundamental possibility of accurately predicting a mutant strain’s occurrence is low, there is extensive research working toward this goal.

**Mutations**

Such mutation is the current issue with a virus whose initial genomic sequence is barely controlled as it is: SARS-CoV-2. Current news reports headline COVID-19 variants detected in the United States. This is not surprising though, at least not to those who have considered the nature of the virus’ genome. As a single-stranded, non-segmented, positive-sense RNA genome, SARS-CoV-2 is highly susceptible to mutations. It should be acknowledged that a mutation does not necessarily equate to a significant change in the virion’s infectious or symptomatic properties. Only those affecting major viral proteins will generate new properties that provide greater benefits to the virus. It is also important to note that although SARS-CoV-2 is similar to other family members of *Coronaviridae*, the viruses cause entirely different diseases because of their acute variances. As mentioned previously in the detailed discussion of SARS-CoV-2’s genomic structure and infection mechanism, this virus employs a lytic replication cycle which requires it to bind and enter the host before releasing its genomic contents. The
human body has several levels of defense which will be triggered almost immediately but ramping up a full-blown immune response takes time.

The Immune Response

The human immune response is a highly evolved system, consisting of organs and various cell types. Tissues and lymph are our primary immunological organs. Cells within these organs have roles to alert the next level of defense to help combat the infection. Macrophages and dendritic cells (DC), both phagocytes, are found in tissues and serve in the innate immune response. Their primary duty, mainly DCs, is acting as an antigen-presenting cell (APC), meaning they will engulf the virion, process it, and show the next responders (T cells, adaptive
response) what they are looking for to ensure they target the correct molecule. Upon activation, B cells transform into plasma cells and begin to secrete antibodies (Figure 9) from within the lymph that travel to the site of infection and employ effector functions against invading virions. Neutrophils are signaled to enter infected tissues from the blood, and they begin engulfing and destroying microbes until they run out of space or energy then will die. Helper T cells (CD4) receive information about the infection from the APCs and exert effector functions through receptors/ligands and cytokines. These cells travel to the site of infection and direct other cells on what to do and where to go. Cytotoxic T cells (CD8+) must also receive direction from an APC, then they will travel to the infection site and seek out all other cells that express the same major histocompatibility complex (MHC)-antigen combination used to activate it. After engaging the antigen-embedded MHC molecules, CD8+ are stimulated to transform into cytotoxic T lymphocytes (CTLs). CTLs directly attack and kill infected cells via apoptosis as a means to prevent further spread of infection. The lymphocyte cannot, however, separate viral material from human, so the entire cell and its contents are destroyed in an attempt to wipe out the microbes. Because the virus is using the host cell’s machinery, it can be difficult for the immune system to determine what is normal and what is infected; it is better to decimate it all and recover later.

The Replication Cycle

Influenza and SARS-CoV-2 are able to reroute human cell organelles for viral processes because human cells use the same genomic material (RNA) to reproduce themselves. The central dogma of molecular biology is the most basic explanation of how human cells replicate their genome. Helicases separate double-stranded DNA into single-
stranded segments which are then transcribed by DNA-dependent RNA polymerase into complementary positive-sense mRNA segments. The mRNA is decoded by transfer RNA (tRNA) molecules within the ribosome where RNA is translated to synthesize a protein from the encoded amino acid sequence. The protein sequence can be used for various functions depending on the cell's origin, including cell effector functions, new cell synthesis, or cell maintenance. Positive-sense vRNA of SARS-CoV-2 is very similar to a human cell's mRNA, thus can be directly translated upon entry into the host cell.

Negative-sense viruses, like influenza, typically bind to the host's plasma membrane by interacting with multiple adjacent membrane receptors to form multivalent interactions, which are essentially multiple weak ligand-receptor bonds (Müller et al., 2019). Therefore, changing the number of linkages changes the strength of the bond. This feature is used both ways—a strong initial attachment enables the next step (viral entry) and a weakened bond allows progeny viruses to leave the membrane after completing replication (Müller et al., 2019). Once inside the cell, SARS-CoV-2 faces fewer obstacles when replicating than influenza viruses do. SARS-CoV-2 spike proteins have a high affinity for binding ACE2 residues; this interaction mediates viral entry and is one of the prime immunization targets (Sanda et al., 2021). ACE2 is a carboxypeptidase that can induce vasodilation by cleaving angiotensin II (Hikmet et al., 2020). The spatial localization of ACE2 on the protein level across the entire human body is still poorly understood but necessary for determining how the expression on the mRNA level can be translated to functional use (Hikmet et al., 2020). Understanding the virion's target should make the virus more predictable.
**Vaccination Overview**

Effective medications and vaccinations rely on accurate understanding of the molecular details of virus structures and infections. Vaccinations are typically an injection of a protein synthesized with pieces from an inactivated bacteria or virus. Thus, vaccinations are only effective in helping the body mount a defense against an infection with that particular microbe. Even then, natural infections oftentimes do not present the exact variant that the vaccine was produced with, so there is still a level of susceptibility. This trend is especially prevalent with yearly influenza strains and vaccines. Antibodies against surface HA glycoproteins neutralize a virion’s infectivity, so virus strains evolve frequent amino acid changes at the antigenic sites while the stem-head configuration of the molecule remains conserved across strains and subtypes (Figure 5) (Bouvier and Palese, 2008). Cumulative adjustments result in a strain that is no longer effectively neutralized by human antibodies against the parental virus, leaving hosts immunologically naïve to productive infection (Bouvier and Palese, 2008). It is worth noting, however, that two of the leading COVID-19 vaccines approved in the United States – Moderna and Pfizer – rely on a new mRNA-based technology and deliver mRNA molecules instead of proteins. The efficiency and sustainability of this type of vaccine has not been tested long-term, so it is difficult to say whether this delivery will become more common. Vaccinations are not effective against various viruses – for the purposes of this paper: the influenza vaccine neither cross-reacts with SARS-CoV-2 proteins in a practical manner nor prepares the body to deal with a SARS-CoV-2 infection. There may be numerable parallels between the influenza virus and SARS-CoV-2, but they must be approached as separate entities.
CHAPTER SIX

Summary and Conclusions

The overarching theme of all the literature that was reviewed is that while influenza and SARS-CoV-2 share certain characteristics, they must instead be defined by their differences. Influenza is a well-observed phenomenon; its annual occurrence and symptomatic presentation is predictable, yet modern medicine still lacks a comprehensive understanding on how to combat it as antivirals lag behind the times. This lack of successful medication can be explained by virus’ advanced infection and replication mechanism that exploits healthy cells, essentially forcing them to sabotage the body by producing more infectious agents. A small number of functional and structural proteins in SARS-CoV-2 that may serve as the target of an antiviral also pose significant challenge for drug design. Both influenza and SARS-CoV-2 – the enveloped, single-stranded RNA viruses causing the flu and COVID-19, respectively, exercise a lytic replication cycle, though with some notable differences. SARS-CoV-2’s characteristic spike proteins grant the virus some unique features, including high infectivity and virulence, both of which made the virus quickly become relevant and demand the world’s attention. While 2020 dragged on as the longest year for various reasons, the severe lack of answers and explanations only served to lengthen the process. There were several questions that had shaken both the general public and the science community – questions that have yet to be answered even today. It will take years of retrospective analysis and research to fully understand the fundamental concepts behind some of the anomalous qualities observed
through infections. The influenza virus has been a valuable tool for comparing and contrasting in order to understand and combat SARS-CoV-2, but the similarities of the two should not be overstated; their differences more holistically define them.

Nonetheless, these joint understandings, along with modern understanding of the human immune response, propelled the fastest vaccine timeline in human history – a notable achievement. Now we are left to process the masses of data, information, and research that came from this overwhelming experience that led to personal and communal turmoil for so many.

**Outlook**

Finding the silver lining in the current economic and political state that the world has found itself in is an arduous, if not impossible, task at times. Yet this situation is not entirely without hope. There is something to be said about the speed of the COVID-19 vaccination production – the likes of which has never been seen before. There is promise moving forward with all that the science community has and is still currently learning about infectious disease, including population spread, mechanism of infection and replication, genomic recombination, antiviral targeting, zoonotic disease origins, and more.

This momentum in the scientific community should be channeled toward creating more effective vaccines. Researchers were forced to dig to a deeper level of understanding of viral genetics than ever before when we were faced with the first novel virus of pandemic proportions in over a century. Influenza was one of these known models. All the knowledge that came with the searching should not be put to the wayside and simply overlooked when a similar event occurs again. That knowledge should be
used proactively to prevent another global tragedy. Vaccinations are the first step in these defensive measures. The strongest, fastest, and most intelligent technology is more readily available to scientists than ever before in human history. Perhaps mapping a timeline of all recorded influenza variants could expose a pattern of genome segment recombination or maybe the binding affinities of various glycoproteins in conjunction could pave the way to accurate predictions of how the next year’s strain will have mutated to achieve immunological susceptibility within its host. The 2020 pandemic forced the scientific community to acknowledge its shortcomings in the decades-long war that is being waged against infectious diseases. A virion that is only 20-500 nm wide effected change on a global scale. It forced us to redefine normal on an economic, personal, and political level and reevaluate how the healthcare system can better respond to novel viruses in the future. Things may never return to how they were before, but that is simply a testament to the way society and science should continue to mutually evolve.
Bibliography


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