Mathematical Modeling for the Assessment of the Pandemic Potential of Influenza A: the Present State and Future Possibilities

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B.S. in Biochemistry and Molecular Biology, April 2011, University of California, Davis

A Thesis submitted to

The Faculty of
The School of Engineering and Applied Science
of The George Washington University
in partial fulfillment of the requirements
for the degree of Master of Science

May 19, 2013

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Acknowledgements

The author wishes to acknowledge Dr. Royce Francis and Dr. Amber Smith for their guidance in developing this thesis. Their insight and assistance has made this a stronger paper.

Executive Summary

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Due to the emergence of influenza A subtype H1N1 in 2009 and H5N1 in 1997, the public is becoming increasingly aware of the dangers of pandemic influenza. A recent, heavily publicized series of experiments performed by researchers at the University of Wisconsin-Madison and at the Erasmus Medical Center in Rotterdam acutely emphasized this threat. The two teams used different methods to mutate the highly pathogenic H5N1 virus, which is currently not transmissible between humans, into a strain that can easily spread from person to person. They discovered that only five mutations are needed for this change to occur and concluded that H5N1 poses a risk for human pandemic influenza (1-3). It is therefore more important now than ever that the mechanisms that underlie H5N1's virulence and transmission capabilities are better understood.

Mathematical modeling is an innovative means of analyzing biological systems, including influenza A infection. The work that has been done so far in influenza A modeling has revealed many important insights into the mechanisms by which the virus operates. Modeling has shown, for example, that new viruses to which the host has no protection are not the most dangerous – those that are only partially recognized by the host immune system are. Competition between mature immune effector cells that recognize the antigen and immature cells that are undergoing the development process result in a suboptimal immune response and more severe disease. Influenza A modeling has not, however, successfully captured the dynamics of viruses with virulent pandemic potential. I define this category of virus as having the following traits: increased virulence over seasonal influenza strains, the ability to transmit human-to-human, and being new to the population (no or limited cross-protectivity). Viral strains that fall under this category interact with the host immune system in a way that is different from seasonal strains. They repress host immune effector cells more successfully, replicate more quickly and elicit a different immune response, and some bind to different host cellular receptors. In order to capture these dynamics, certain modifications and additions need to be made to current models. Suggestions for some of these changes are provided, while others are more complex and require additional research.

This paper highlights the features that are lacking in current mathematical models with regard to highly pathogenic viruses. It also points out the aspects of these models that can be joined and modified to create the 'ultimate' model – one that predicts the pandemic potential of a new strain. This model would include three major features: virulence, transmission, and cross protectivity. Virulence would be an attribute that encompasses all of the known factors that affect pathogenicity, all of which are discussed in a later chapter. A set of threshold points would be established for various values within the model, such as the rate of change of the host cell population and the viral replication rate. Subtypes which exceed these values would be classified as virulent, while others would not. A similar approach would be taken for the transmission and cross protectivity pieces of the model. This method would allow the modeler to establish a way of quantitatively predicting the virulent pandemic potential of a particular subtype. The final model output would be a measurement that indicates how dangerous the subtype is and therefore guide public health officials in preparing against a potential pandemic. Many of the equations and variables necessary to create this model have already been developed, they are simply present in separate models. By joining them into one and making some of the modifications suggested, the creation of the ultimate predictive model is possible.

Of course, the limitations of current technology and the gaps in knowledge of influenza A biology that would hinder such an endeavor are daunting. However, given the impact that such a model would have on our knowledge of virology, mathematical modeling of biological systems, and global health, it is an endeavor that would be well worth the effort.

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List of Symbols/Nomenclature

1.	T	Susceptible cells
2.	B	Infection rate
3.	V	Infectious viral titer
4.	I_1 or E_1	Infected cell population not producing virus
5.	I_2 or E_2	Infected cell population actively producing virus
6.	k	Rate of transition from I_1 to I_2
7.	p	Virus production rate
8.	c	Virus clearance rate
9.	δ	Infected cell death rate
10.	F	IFN titer
11.	s	IFN is production rate
12.	q	IFN production rate in different model
13.	α	IFN clearance rate
14.	T	Time IFN begins to be produced
15.	ϕ	Rate that IFN induces an antiviral state
16.	W	Prerefractory cells
17.	R	Refractory cells
18.	a	Rate of transition between W and R
19.	X	Adaptive immune cells
20.	f	Recruiting rate of the adaptive immune cells
21.	r	Expansion rate of adaptive immune cells

List of Acronyms

1.	HA	Hemagglutinin
2.	NA	Neuraminidase
3.	NS protein	Non-structural protein
4.	RNA	Ribonucleic acid
5.	GTP	Guanosine triphosphate
6.	IFN	Interferon
7.	TFN	Tumor necrosis factor
8.	IL	Interleukin
9.	TGF	Transforming growth factor
10.	CTL	Cytotoxic T-cell
11.	NK cell	Natural killer cell
12.	SA	Sialic acid
13.	M	Matrix
14.	HIV	Human immunodeficiency virus
15.	HCV	Hepatitis C virus
16.	WHO	World Health Organization

Glossary of Terms

Mathematical model: a description of a system using mathematical concepts and language that can be used to explain and study that system.

Virus with pandemic potential: a virus subtype which can possibly initiate a pandemic as defined by the World Health Organization: "Phase 6, the pandemic phase, is characterized by community level outbreaks in at least one other country in a different [second] WHO region in addition to the criteria defined in Phase 5. Designation of this phase will indicate that a global pandemic is under way"

Virus with virulent pandemic potential: a virus subtype which possesses all of the following traits: increased virulence over seasonal influenza strains, the ability to transmit human to human, and being new to the population.

Highly pathogenic virus: a virus with the ability to produce significant disease.

Viral serotype: a viral strain distinguished by a shared set of antigens.

Virulence: the degree of pathogenicity. The extent to which a particular viral strain causes disease.

Human-human transmission: the ability of a virus to be passed directly from one person to another by either aerosol or contact routes.

Cross protectivity: that is the reduction in susceptibility to serotype A conferred to a host by infection with serotype B.

Antigenic distance: the genetic variation between serotype A and serotype B.

Chapter 1: Introduction

Influenza A is a highly infectious viral disease that causes annual epidemics and occasional, sometimes devastating pandemics. The 1918 Spanish flu pandemic resulted in over 50 million deaths and, according to experts, another influenza A pandemic in the near future is a virtual certainty (7). Numerous advances in research and treatment methods have been made in recent decades but still, many questions remain unanswered. Why, for example, do the 1918 H1N1 and highly pathogenic H5N1 viruses fatally infect an unusually high proportion of young individuals? What combination of mutations results in a virus with pandemic potential? A relatively new, potentially revolutionary method for answering many of these questions is mathematical modeling. A simplified model of infection boils down the problem to the essential variables and allows the researcher to test theories and draw new conclusions. For instance, a model can demonstrate that mutations in the viral NS1 protein result in a virus with increased replication rate and therefore increased pathogenicity. The challenge lies in developing a model that accurately encompasses all relevant biological traits and is still simplified enough to be considered a model.

One important feature that I find lacking in current influenza A mathematical models is the inclusion of characteristics which represent highly pathogenic strains. Most models that have been created to date attempt to capture the dynamics of seasonal influenza strains, not those that are highly pathogenic. They are based on data from low mortality viruses such as equine influenza which exhibits different characteristics from a virus with pandemic potential. With new viruses such as the 2009 H1N1 'swine flu' and 1995 H5N1 'bird flu' emerging as potential instigators of a worldwide outbreak, it is becoming increasingly important to develop the ability to evaluate a new serotype's pandemic potential. Current mathematical models do not have the features necessary to carry out this assessment, but if they did, they would be an invaluable tool in pandemic preparedness.

My objective in this paper is to review the extent to which current models accurately capture the features of highly pathetic viruses and examine the possibility of creating a model that assesses the pandemic potential of a specific strain. I begin with an overview of basic influenza A biology and a review of the known determinants of viral pathogenicity. Viral pathogenicity can be divided into two categories: those characteristics which contribute to viral transmission and those which contribute to virulence. I then review all influenza A models developed to date, focusing on each models' potential usefulness in

assessing a highly pathogenic strain. Some models are more extensive than others and many contain features that could be included in an all-encompassing model (39-42, 44, 48). Finally, I discuss the possibility of creating the ultimate model – one that has the ability to assess the global impact of a new influenza A strain. I outline the features that I think should be included in this model and discuss the challenges that hinder its creation. The public health benefits that would result from the development of such a model are enormous. It is to the advantage of researchers and health officials alike to continue to make progress towards the eventual creation of the ultimate influenza A mathematical model.

Chapter 2: Background Information

2.1 Influenza A Biology

Influenza A is an acute viral infection that results in three to five million severe cases yearly and 250,000-500,000 deaths. Seasonal epidemics, which occur annually during the winter in temperate regions, are a serious public health problem for higher risk populations and can have severe economic consequences. Currently, subtypes H1N1 and H3N2 are circulating among humans and are therefore included in seasonal influenza A vaccines (4).

The influenza A virus consists of 11 proteins enveloped by a host-derived lipid membrane. Each protein carries out a specific functional role that is essential to the infection process (75). Hemagglutinin (HA) is the main surface antigen present on the viron surface and is the major target of the host immune response. The HA protein mediates viral entry into the cell by attaching to the host cell sialic acid receptor which initiates receptor-mediated endocytosis and draws the viral particle into the cell. Viral exit from the cell is in turn carried out by the second major surface antigen, neuraminidase (NA). This protein cleaves the bond between sialic acid and glycoproteins, allowing viral progeny to escape the cell. Both HA and NA have high mutation rates which generate variants such as H5N1 and H1N2. Mutants that are not recognized by the host immune system are especially infectious and have the potential to create a pandemic (75).

The viral polymerase is composed of the proteins PB2, PB1, and PA. These three proteins are present as a complex in the complete virus particle and together enable the virus to initiate transcription of viral RNA after infection. Each of the three proteins carries out a specific function in transcribing newly synthesized RNA (75). The nucleoprotein (NP) interacts with the viral polymerase complex to facilitate RNA replication. It is a protein with a highly conserved sequence that is produced abundantly in an infected cell and functions by providing a binding site for the RNA polymerase to the RNA molecule (74). Matrix protein M2 is present in large amounts on the viral cell surface and is believed to serve as a proton channel protein that controls the pH to allow acidification of the interior of the viron during virus uncoating. M2 also serves as a signal protein for transport to the cell surface. Matrix protein M1, on the other hand, does not have enzymatic activity and instead plays a role in the assembly of virus progeny. Nonstructural proteins NS1 and NS2 further contribute to viral replication (74). NS1 in particular has been

shown to interfere with host transcription and nuclear exportation of mRNA to allow for preferential processing of viral RNA (76).

Current antiviral drugs used to treat influenza A target NA, M2, and cellular proteins because they are either present on the viral surface or within the host cell. Oseltamivir, marketed as Tamiflu, and zanamivir interfere with the NA protein, blocking the virus' ability to leave host cells and therefore preventing the spread of the virus. Amantadine inhibits the virus from uncoating once it enters the cell by hindering the M2 channel ion protein, while ribavirin blocks the function of a cellular enzyme by disabling the synthesis of GTP and viral RNA. These drugs can be used either alone or in combination to treat influenza A (52). They have a short window of effectiveness that is usually limited to two days after infection, thus presenting a challenge for treatment (57). Research aimed at discovering new antiviral drugs is currently focusing on the M2 and NA proteins as well as HA, NS1A, NP and the viral polymerase. Seasonal strains and H5N1 have developed resistance to several known antiviral drugs, including amantadine and oseltamivir, necessitating the development of new drug candidates (59, 110). The mutations that confer drug resistance are known and, depending on the viral protein target, can develop rapidly. For example, the amino acid substitutions that grant a virus amantadine resistance are not present in nature, but they occur in up to 80% of people treated after only a few days of therapy (111). The single amino acid substitution H274Y that affords H5N1 oseltamivir resistance is not as common but may be associated with increased mortality (110). As the number of serotypes circulating within the human population grows, so does the need to for antiviral drugs with diverse targets.

Influenza A preferentially infects epithelial cells in the respiratory tract, but has also been shown to infect macrophages and natural killer cells (5, 6). Invading virus particles attach to sialic acid receptors on the cell surface and enter the cell through endocytosis. Inside the cell they create a low pH environment which allows the viral particle to shed its exterior, releasing viral protein and RNA which then migrate to the nucleus to begin replication. Viral RNA is produced in the nucleus, translated into protein in the Golgi, and then assembled in the cytoplasm. Newly assembled virus particles are released from the cell through budding at the host cell membrane (74, 77). This entire process, from infection to viral release takes 5 to 6 hours and peaks after 7 to 8 hours. The delay in production of viral progeny after infection is called the "eclipse phase". Viral proteins released from one cell will go on to infect another, exponentially increasing

viral load until the maximum is reached around 3 to 5 days post infection (10). It takes an average 2 to 4 days for a host to become infectious. At this point, the infected person has a high enough viral load to begin spreading the disease to others through airborne particles or contact (109). Infection is cleared through the body's immune responses.

Most virons that enter the respiratory tract are cleared through non-specific interactions without the help of the immune system (10). Those that do bind to target epithelial cells activate the innate immune system which rallies alveolar macrophages, neutrophils, and natural killer cells (NK cells) to fight the invaders. These cells appear on the scene immediately, excreting cytokines, messenger molecules of the body, the most important of which are type 1 interferon (IFN-α/β), interleukin-1α/β (IL-1α/β), IL-6, IL-8, and tumor necrosis factor-α (TFN-α) (9). IFN-α/β help limit viral replication while interleukins and TNF-α activate NK cells. NK cells in turn excrete IFN-γ, a cytokine that aids in destroying virus infected cells (8). Cytokines also activate the adaptive immune system, which fully kicks in about 3 days post infection. The effectors of the adaptive immune system, virus-specific antibodies and cytotoxic t-cells (CTLs), then clear the infection entirely, leaving behind memory B-cells that provide protection against subsequent infections (8).

The immunological memory gained after recovery from an influenza infection does not protect against new serotypes of the virus. These emerge as a result of the high mutation rates of influenza A that lead to mistakes in the viral genome and new viral strains, termed antigenic drift, and through reassortment of different serotypes in an animal host, termed antigenic shift (11, 12). Influenza A has a high mutation rate due to mistakes made during RNA transcription. The mutations that lead to amino acid changes in the HA and NA proteins can result in new forms of the virus to which humans have no preexisting immunity. Immunological memory cells such as antibodies would therefore have no effect against this virus. Antigenic shift is the change in viral specificity due to recombination in an animal host (12). The virus can be transmitted directly from an animal reservoir to a human or it can arise as a result of reassortment between human and animal viruses (13). The 16 subtypes of HA and 9 subtypes of NA that currently exist in nature can be found in a wide variety of animals such as waterfowl, pigs, leopards, seals, and whales, although the main influenza A virus reservoir is birds (12). Several serotypes of influenza A, including H5N1, are endemic to wild birds and for the most part, are asymptomatic in them just as certain serotypes

are endemic to pigs (13). These viruses circulate and mutate within their animal host populations and then spread to humans as a result of antigenic shift, possibly creating a pandemic as in the 1918 Spanish flu or 2009 H1N1.

The main barrier to avian-human virus spread is difference in receptor specificity. Avian viruses bind to sialic acid receptor of type α(2,3) while human and swine viruses bind to the α(2,6) variant (13). Pigs harbor both types of receptor specifies and are therefore considered a "mixing vessel" for influenza A. They can bind viruses of pure avian origin and those that circulate among humans and then allow them to recombine into a serotype that poses a greater danger to humans with regard to contagiousness and virulence (11). Cells with SAα(2,3) specificity can also be found in the human lower respiratory tract, but they are less common than the SAα(2,6) receptors found primarily in the upper respiratory tract.. This could explain why avian viruses such as H5N1 are able to cross over the species barrier and infect humans but they cannot be transmitted between humans. A virus that replicates in the upper respiratory tract is readily spread through coughing and sneezing while one localized in the lower respiratory tract is not (14).

2.2 Determinants of a Pandemic Subtype

The World Health Organization (WHO), the primary authority for health within the United Nations system, does not define what constitutes a virulent pandemic influenza subtype, nor does any other major health organization. The most that the WHO offers is a description of an influenza pandemic situation as a sustained outbreak of a virus at the community level in at least two WHO regions. It states that the virus must be a "new influenza virus against which the human population has no immunity" and must be capable of human-human transmission (90). This description was the source of some controversy in 2009 when the WHO declared a pandemic situation for the H1N1 subtype. Many countries mounted aggressive responses in preparation for the pandemic, but found that it had far less serious consequences than experts predicted (91). The lack of a definition of pandemic influenza therefore came into question. A concrete definition of the biological characteristics that constitute an influenza subtype with pandemic potential would be very useful in avoiding situations such as the one described previously. Here I define a serotype with virulent pandemic potential as having the following traits: the ability to transmit human-to-human, increased virulence over seasonal influenza strains, and being new to the population (no or limited cross-protectivity).

The first two criteria are influenced by a number of factors, many of which are still unknown, while the last is a simple matter of antigenic distance. Viral pathogenicity increases as the difference between the genetic sequence of the virus and the sequence that the host immune system recognizes increases. The less recognizable or more new a virus is, the more virulent it will be because the host cannot eliminate it as quickly (112). The other two criteria, transmission and pathogenicity, are described in more detail in the following section as there are a number of elements that cause them to differ between subtypes.

2.2.1 Transmission Determinants

Receptor specificity and human-human transmission of H5N1 was the main focus of the experiments performed by the two teams at the University of Wisconsin-Madison and at the Erasmus Medical Center in Rotterdam. Immunologists at Erasmus Medical Center discovered that four mutations, three in the HA protein and one in PB2, were all what was necessary to create a virus that was airborne transmissible in ferrets. The team at the University of Wisconsin-Madison created a recombinant virus with a mutant HA from H5N1 and the remaining genes from H1N1 that was capable of transmission in ferrets. In both studies, the mutations in HA caused a receptor binding switch from SA α(2,3) to SA α(2,6), suggesting a human-type receptor preference is necessary for airborne transmission. The mutations also resulted in a less pathogenic form of the virus. None of the ferrets infected with the mutant virus died. The authors of these articles were able to conclude that H5N1 can acquire the ability to transmit between human hosts without recombination in an intermediate host and therefore constitute a risk for a human pandemic influenza (1-3).

However, binding to SAα(2,6) and reduced virulence are not definitively correlated. The 1918 H1N1 pandemic virus that was extraordinarily lethal preferentially recognized SAα(2,6). When it was mutated to recognize only SAα(2,3) through two amino acid changes at positions D190E and D225G, it lost its ability to transmit between ferrets but remained highly pathogenic. Interestingly, the mutant virus with avian specificity could replicate at high rates in the upper respiratory tract but it did not induce sneezing in ferrets like the transmissible virus (16). Amino acid substitution sites that confer avian-human receptor specificity differ between HA subtypes. The sites mutated on the HA protein by the team at the Erasmus Medical Center were different than those mutated on the H1 subtype and H2 and H3 subtypes in other

experiments (1). These results indicate that receptor binding to SAα(2,6) determines transmission capabilities but not virulence (1-3, 14). They also demonstrate the high variability between influenza A serotypes.

In addition to preferential human receptor binding, a mutation in the viral polymerase protein PB2 is also necessary to facilitate inter-host transmission and human adaptation (19, 20). A single amino acid substitution at position 627 (Glu to Lys) is essential for efficient replication of influenza A viruses in human cells. All avian viruses possess glutamic acid at this position, while all human adapted viruses possess lysine (17). A 1918 H1N1 virus possessing an avian (Glu) PB2 protein is not capable of airborne transmission while the wild-type virus (G627K) is (19). In mice, this mutation occurred naturally after a single passage in naïve mice and it led to more aggressive virus replication and increased lethality (18). The G627K mutation is the only known mutation that is essential for the adaptation of avian viruses to human hosts and for human-human transmission (17-20, 93, 94).

Table 1: Determinants of airborne viral transmission

Protein	Requirement	Role in transmission	Reference
HA	Binding preference to SAα (2,6)	Necessary for airborne transmission of virus	1-3, 14
PB2	Mutation G627K	Essential for efficient viral replication in human cells	17-20, 93,94

2.2.2 Virulence Determinants

H5N1 and 1918 H1N1, both extraordinarily virulent strains of influenza A, are set apart by high chemokine and cytokine levels and high viral replication (30, 31, 33-35). The high levels of pro-inflammatory compounds and high viral titers that they induce are not seen in less pathogenic influenza A viruses or those responsible for the seasonal flu. This might explain why younger individuals, who generally have stronger immune systems, were infected at abnormally high rates by H5N1 and 1918 H1N1 (31). TNF-a, IFN-B, and IL-29 are particularly potently induced by H5N1 infection versus seasonal H1N1 infection. TNF-α and IFN-β have been shown to act synergistically in up regulating host pro-inflammatory cytokine responses. They are up regulated more than 1.5 times in magnitude during H5N1 infection over seasonal H1N1 infection, which likely leads to the hypercytokinemic response and tissue damage seen in patients

(38). Replication rate is also a major player in lethal H5N1 infection. The virus exhibits extremely high replication rates in comparison to less virulent influenza serotypes. A recent study indicated that replication rates, not an aberrant cytokine response, might be the main reason for the exceptional lethality of H5N1. Mice deficient in pro-inflammatory cytokines were infected with H5N1 and still succumbed to the virus, but those with decreased viral replication exhibited less mortality (36). Both factors set highly pathogenic strains apart from their less lethal counterparts and therefore must both be examined as causes of increased virulence. Two factors that significantly contribute to hypercytokinemia and high replication rates are described below.

The role of apoptosis, or cell suicide, in influenza A infection is complex and especially significant when considering the activity of highly pathogenic strains. While the induction of apoptosis is necessary for efficient viral replication and spread, the NS1 protein is known to play a role in preventing apoptotic cell death (64). Experimental evidence suggests that the prevention of early apoptosis is advantageous to the virus (64), most likely because the virus is allowed to replicate within host cells for a longer period of time which increases the overall viral load more quickly. In ducks infected with viruses of varying pathogenicity, only lethal H5N1 failed to induce rapid cell death (64). The correlation of suppressed apoptosis and increased virulence persists in other studies as well (65, 66).

A proposed mechanism for the prevention of apoptosis in cells infected with highly pathogenic strains is the suppression of transforming growth factor beta (TGF-β). In two comparative studies between virus subtypes, researchers found that the viruses that exhibited the highest levels of pathogenicity also failed to activate TGF-β, a cytokine that plays a role in inducing apoptosis and both induces and resolves inflammatory responses (60, 62). When TGF-β was given to mice infected with lethal H5N1, researchers saw delayed mortality and reduced viral titers. This data indicates that TGF-β plays an important role in protecting the host during infection and highly pathogenic serotypes have developed strategies to prevent its activation (62). Furthermore, the NA protein is known to directly stimulate the production of TGF-β (61). While all of the afore mentioned studies were performed on highly pathogenic H5N1, 1918 H1N1 and H5N1 viruses share a 92% homology in their NA protein sequences (63), indicating that the structure of the NA protein and its ability to restrict production of TGF-β is likely a determinant of pathogenicity.

The NS1 protein is also an important virulence determinant in influenza A viruses. NS1 proteins differ between subtypes and cause varying levels of cytokine/chemokine induction and apoptosis in epithelial cells. The NS1 protein enables the virus to down regulate IFN production, resulting in a more lethal virus (21, 24). In the case of H5N1, NS1 activity is extreme, possibly resulting from a mutation at position 92 that is only present in lethal forms of the virus. H5N1 possesses a gluatimic acid at this position while other known influenza viruses possess an aspartic acid (100). The NS1 protein coded by the 2009 pandemic H1N1 strain induced cytokine levels comparable to that of seasonal H1N1, while the NS1 protein in H5N1 induced very high cytokine expression (21). Mutant viruses containing an NS1 sequence from 1918 H1N1 and H5N1 exhibited significantly increased virulence over their wild-type counterparts in the form of greater lung damage and viral spread. Researchers found that this is most likely the result of a modified C terminus sequence in highly pathogenic strains (23). The NS1 gene from the 1918 H1N1 virus is particularly effective at blocking the cells' IFN pathways and therefore reducing their ability to fight infection compared to less virulent strains (22, 73). The NS1 protein manages to block expression by using a histone-sequence and hijacking host proteins to suppress gene expression of the antiviral response (32).

PB1-F2 has similarly been implicated in the pathogenicity of influenza A. It delays viral clearance and increases viral pathogenicity but does not have an impact on viral replication (25 -29). PB1-F2 proteins derived from three pandemic strains and H5N1 all showed enhanced cytokine production and virulence in contrast to less virulent strains (26). A polymorphism at position 66 in PB1-F2 that is present in 1918 H1N1 and some H5N1 isolates definitively increased pathogenicity of the virus over other strains (28, 29). A mutation in this position also reduces the induction of IFN, leading to an increase in disease severity (29). The virulent effects of PB1-F2 appear to originate in birds and then disappear in mammalian species over time. The PB1-F2 proteins from human-adapted less virulent strains do not exhibit the same level of pathogenicity (26, 27).

The HA protein must be posttranslationally cleaved by host proteases in order to become biologically active. The susceptibility of HA to these proteases differs between strains and has been shown to influence viral pathogenicity. A virus with an HA protein that is sensitive to a wide variety of proteases is not only more easily cleaved and therefore is able to replicate more efficiently, but it also enables the virus to spread systemically (15). If the HA can be cleaved by any number of proteases, then its range

within the host can extend more easily to include the inner layers of respiratory and intestinal organs, leading to eventual penetration into blood vessels. Viruses that reach the blood can establish a dangerous, systemic infection that can lead to death (69). In pathological studies involving H5N1 victims and ferrets, the virus was found in the lungs and intestines as well as the bone marrow, neutrons of the brain, and other organs beyond the respiratory system indicating that the virus has a wide tissue tropism. Virus replication, however, was limited to the respiratory tract (85, 86). Conversely, 2009 H1N1 was typically contained to the respiratory system in a study done of 100 fatal cases (86). These studies done on influenza A tissue tropism support the assertion that extremely virulent viruses exhibit high cleavability, while milder strains have a restricted cleavability (68-70). Interestingly, all avian viruses lethal to humans possess a highly cleavable HA and have a cleavage site with a string of basic amino acids (69). Viruses that are mildly pathogenic or of non-avian origin, like 2009 H1N1, possess an arginine residue at the cleavage site that restricts viral replication to the respiratory tract (15). For H5N1, the HA protein is virulence marker (70-71). A wild-type HA results in a highly pathogenic form of H5N1, but its virulence is attenuated when the HA protein is modified (70). This underscores the importance of HA cleavability in determining the virulence of a specific strain.

Influenza A pathogenicity is clearly a polygenic trait. While all of the above are considered virulence markers, more than one is required to instigate a change in pathogenicity. For example, a mutation at position 627 is generally accepted to be an indication of virulence (17-20), but that's not always the case (50). When the mutation is introduced into the H1N1 virus of swine-origin which does not display high levels of virulence, there is no change in pathogenicity or replication rate (50). The NS1 gene of 1918 H1N1 is effective in modulating host cytokine responses, but not alone (72, 73). Without the addition of its own HA, NA, and M genes, the NS1 protein in a recombinant virus does not increase lethality (72). Additionally, high cleavability of HA significantly contributes to the virulence of H5N1, but is not necessary to induce high pathogenicity for 1918 H1N1 (70). All, or at least most, of the viral genes must work in tandem in order to affect virulence (67).

It is therefore necessary that the entire virus phenotype is taken into account when assessing a particular serotypes' potential for virulent pandemic influenza. One cannot rely solely on the presence or absence of a single virulence marker – an entire picture of the virus is necessary. In the next section I

present mathematical modeling as a tool for obtaining this complete picture. I review the mathematical models that aim to capture the kinetics of influenza A infection as a whole and in doing so reveal additional insights into the inner workings of the virus.

Table 2: Determinant factors of viral pathogenicity

Protein	Mutation	Role in pathogenicity	Reference
NS1	C terminus	Increased damage to epithelial cells	23
	Asp92Glu	Cytokine resistance	100
PB1-F2	Asn66Ser	Induction of apoptosis	28
PB2	Gly627Lys	Determinant of replication potential in mammalian cells	17
HA	Polybasic cleavage site	Increased tissue tropism. Other roles unclear	69
NA	N/A	Prevention of premature apoptosis and suppression of TGF-β	60-62

Chapter 3: Mathematical Modeling of Within-Host Influenza A Dynamics

3.1 A Review of Models Developed to Date

One of the purposes of mathematical modeling is to reduce the complexity of a biological system as described in the previous section to a simple, logical model. Assumptions and theories can be drawn from these models which can then be tested against empirical data (37). These models can help researchers understand and quantify the processes involved in disease infection and thus aid in the creation of better treatments and more effective disease management strategies (10).

Over the last two decades, a number of mathematical models have been developed to study the within-host dynamics of influenza A. Many of these models have been recently reviewed in (10, 46). These two reviews focus on analyzing model formulation and compare the models on the basis of structure, parameter estimation techniques, and data sets used. They look at the values obtained by the models and compare them to experimentally determined values to evaluate model accuracy. While both discuss the contributions that each model makes to the understanding of influenza A dynamics, it is not the main point of either review. In contrast, my review of the 16 models developed to date focuses on examining the features of each model that are useful in assessing the virulent pandemic potential of a particular subtype.

3.1.1 Target-Cell Limited Model

The simplest models follow a target cell limited approach and ignore the effects of the immune response. In this model, the invading virus continuously infects epithelial cells until they are depleted to a point where the viral population must also decline. It is, in essence, a predator-prey relationship as described by the Lotka-Volterra model of population dynamics (37). This model was further improved upon by incorporating a delay between infection and viral production. Baccam et al showed that while using a delay feature did not significantly improve the fit of data to the model, it was more realistic. The following four differential equations represent the target-cell limited model with delayed virus production (39).

$$\frac{dT}{dt} = -\beta TV$$
$$\frac{dI_1}{dt} = \beta TV - kI_1$$
$$\frac{dI_2}{dt} = kI_1 - \delta I_2$$
$$\frac{dV}{dt} = pI_2 - cV$$

Susceptible cells, T, become infected at rate βTV where β is the infection rate constant and V is the infectious viral titer. Infectious cells are split into two populations, I_1 and I_2, representing the infected population not producing virus and the population actively producing virus, respectively. A cell transitions from I_1 to I_2 at an average rate of $1/k$. Cells I_2 produce virons at rate p and die at rate δ. Infectious virons are cleared from the system at rate c (39).

Baccam et al were the first to apply this model, initially used to describe HIV and HCV infection, to data from six patients infected with influenza A/Hong Kong/123/77 (H1N1) infection. By using nonlinear least squares regression to fit the data to their model, they were able to estimate the average lifetime of an infected epithelial cell at 11.4h and a half-life for free virons at 3.2h. Infected cells begin producing virus particles after 6h and continue to do so for about 5h (39). This is a simple, fundamental model that can be modified to incorporate additional, more complex aspects of infection. It would likely form the foundation for a model that analyzes highly pathogenic serotypes.

Smith et al used the same data from experimental H1N1 infection as Baccam et al to develop a two-phase solution to the above delay model. They separated viral infection into two phases with two viral titer peaks over the 7-10 day disease duration. Phase 1 is characterized by an initial dip in virus levels followed by exponential growth that lasts until 10% of target cells are depleted. Phase 2 occurs when viral growth begins to slow. Smith et al also indicate that the two phase method can be used to compare different viral strains. If one were to compare strains that differ only in their HA and therefore infectivity (β), one can assume that other parameters are similar and test the behavior of each strain. The pathogenicity and transmissibility could also be estimated by looking at I_2 and V (40). This two phase approach holds potential for assessing the pandemic potential of a strain on a general level. It would be useful to expand the two phase model and incorporate more specific parameters describing the virulence and transmission capabilities of the virus. I_2 and β do not effectively capture the complexity of viral pathogenicity but they do provide starting point for a comparative model that analyzes the pandemic potential of a virus.

The target-cell limited model does not take the immune response into account which, as previously discussed, is paramount to disease progression and recovery. Baccam et al and other researchers

went on to examine the effects of incorporating an immune response in the above model.

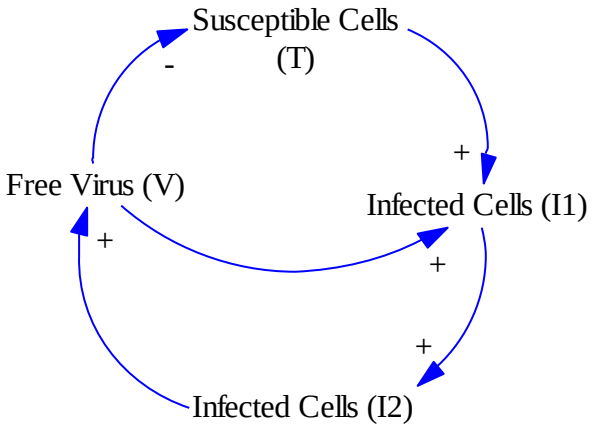

Figure 1: Target-cell limited model of infection. Illustrates the relationships between variables in the target-cell limited model of infection.

3.2.1 Incorporating the Immune Response

The existence of two viral titer peaks in the experimental data used by Baccam et al prompted them to incorporate an IFN response in their model. They added the following equation that models the IFN population:

$$\frac{dF}{dt} = sI_2(t-\mathcal{T}) - \alpha F$$

s is the rate at which IFN is produced, T is the time IFN begins to be produced, and α is the rate at which the IFN is cleared. This inclusion produced a second viral titer peak 36 to 48 h after the initial peak, representing the increased transition time *k* from E_1 to E_2 and reduced viral production rate *p*. They found that a delay of 0.5 days in IFN production was necessary to accurately capture the data. Baccam et al were limited in their model by a lack of data capturing IFN titers in their human volunteers (39).

A later model designed by Saenz et al used viral titer and IFN titers from ponies infected with equine influenza virus data to create a mathematical model. To Baccam et al's equations, they added several parameters. E_1 represents the first eclipse phase or the time before a newly infected cell starts producing virus particles *V*. IFN (*F)* is released by infectious cells (*I*) at a rate of *q* per day. It takes prerefractory cells (*W)* an average of 1/*a* days to become refractory cells, *R*, a state in which they are

primed against viral infection. If during the lag period between *R* and *W* the cell becomes infected, it enters eclipse phase E_2 and secretes IFN at a rate of *nq* per day (41).

$$\frac{dT}{dt} = -\beta TV - \phi FT$$
$$\frac{dE_1}{dt} = \beta VT - k_1 E_1$$
$$\frac{dW}{dt} = \phi FT - m\beta VW - aW$$
$$\frac{dE_2}{dt} = m\beta VW - k_2 E_2$$
$$\frac{dR}{dt} = aW$$
$$\frac{dI}{dt} = k_1 E_1 + k_2 E_2 - \delta I$$
$$\frac{dV}{dt} = pI - cV$$
$$\frac{dF}{dt} = nqE_2 + qI - dF$$

Saenz et al used their model to explore the idea of how a virus that induces high amounts of IFN and simultaneously evades its effects could be particularly detrimental to the host. They found that high levels of IFN induction coupled with decreased IFN efficiency (*dR/dt*) leads to a large percentage of increased cell death (41). The success of Saenz et al in formulating this element of the model is important because it recognizes that the immune system can be detrimental to the host as well as the virus. This is an especially important concept when considering highly pathogenic strains that induce hypercytokinemia. The damage to host extends beyond destruction of epithelial cells and therefore is a more intricate interaction to model. The development of the IFN efficiency parameter is useful in itself and in that it suggest other parameters, like cross-protectivity, can be modeled in a similar fashion.

3.3.1 Incorporating the Adaptive Response

Other models have since incorporated an adaptive immune response. The earliest model to my knowledge that incorporates both the innate and adaptive immune system was created by Bocharov and Romanyuka by applying differential equations written for hepatitis B infection to influenza A infection. Using 60 variables that represented 13 state variables, they found that viral load was more important to disease severity and progression than immune responses. They noted that non-specific and specific immune responses were of

equal importance in viral elimination and a burst of cellular regeneration takes place during infection to compensate for an increase in cell death. Like many modelers, they were limited by a lack of homogenous data sets to fully characterize their model variables (80). Hancioglu et al based their model on the work done by Bocharov and Romanyuka using published experimental data. They performed a sensitivity analysis on model parameters and determined that initial viral inoculum does not affect disease severity, only the onset of disease. Antibodies were found to be essential to recovery. The innate immune response could bring the viral load down to a low level, but antibodies were necessary to clear the infection. It was therefore determined that both the innate and adaptive responses were necessary for recovery (79). The discovery by these modelers that the adaptive immune system, particularly antibodies, is important for disease recovery has important implications for assessing subtypes new to the population. A person infected by a new virus will not have any pre-existing immunity and will likely therefore have a weaker, delayed adaptive response (58). Models that incorporate elements that characterize the time-dependent significance of an adaptive response should be included in a pandemic influenza model.

Handel et al further studied the kinetics of a combined innate and adaptive response using data collected from influenza A infected mice. They created a model that included both innate and adaptive responses, using IFN as a proxy to represent the entire innate immune response. Handel et al added the following equation to their target-cell limited model incorporating an innate immune response in order to represent the adaptive immune system.

$$\frac{dX}{dt} = fV + rX$$

X is the population of adaptive immune effector cells (CTLs and antibodies), f is the rate at which they are recruited to the infection site, and *r* is the rate at which they expand. They used virus-specific antibodies to model their adaptive response but found when it was replaced with CTL dynamics, they obtained the same results. In order to make more detailed models and discriminate between model types, more data and a more complex model would be needed (43). Pawelek et al also came to the conclusion that the inclusion of both responses is necessary in a mathematical model using a different (but similar) set of differential equations than Handel et al. and using data from the equine influenza virus

instead of mouse models. Pawelek et al modeled the effects of the adaptive immune response by changing the infectious cell death rate δ after the adaptive response emerges, while Handel et al included a separate equation for the adaptive response based on the assumption that the adaptive response is proportional to the free virus load (43, 45). The model created by Pawelek et al was sufficient to explain the viral titer pattern that they observed. They saw a major peak in viral load followed by a rapid decline due to the innate immune response and then a plateau or second peak in viral load followed by complete clearance. The second peak and subsequent clearance are the result of the decay of innate responses and the emergence of adaptive responses. However they are unsure whether CTLs or antibodies are responsible for final clearance (45). The two approaches to modeling the adaptive response outlined here both created curves that accurately modeled their data sets, indicating that a more conclusive study is needed to define the best approach. In order to identify differences between influenza subtypes, it is first necessary to understand the role of the adaptive responses. This is an area in need of additional research.

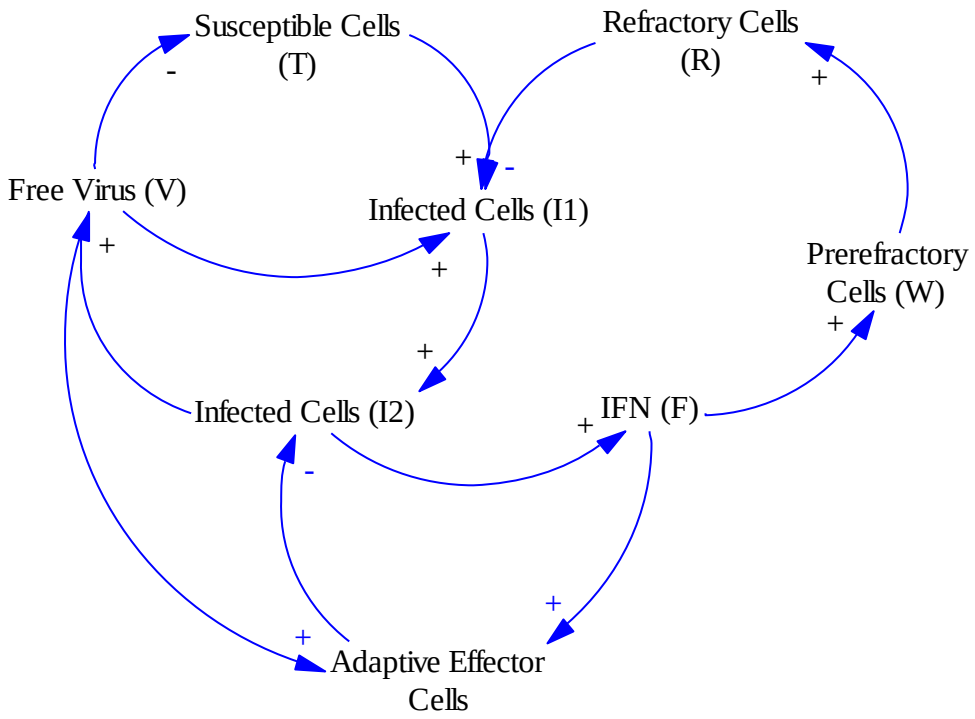

Figure 2: Target-cell limited model incorporating innate and adaptive responses. Indicates the inhibitory or enhancing effects of components of the innate and adaptive immune system on the target-cell limited

model.

Models by Chang et al and Tridane et al focused specifically on the CTL response. Tridane et al designed two models based on the target cell limited model that were able to capture the behavior of CTLs at high density but failed to simulate appropriate viral clearance dynamics. Chang et al also included the IFN response in their model and showed that the initial viral titer peak is controlled by the innate response and the final clearance of viral particles is determined by CTLs (49, 78).

A more detailed model outlining the adaptive response that also included antigen presentation was put forth by Lee et al. They separated the model into two compartments representing pre and post adaptive states and expanded the model to represent the effects of dendritic cells, CD4 T-cells, CD8 T-cells, and both IgG and IgM antibodies. They discovered that the response of antigen presenting cells (APCs) to the virus significantly affects disease duration (48). A year later, Miao et al used data from 340 mice infected with H3N2 to validate this model. In phase 1, they attributed infectious cell death to immune and natural factors, but did not specifically include an IFN or other cytokine response. In phase 2, when the adaptive response appears about 5 dpi, they saw that CTLs had the greatest effect on viral clearance, reducing the half-life of infected epithelial cells from 1.16 to 0.52 days. They also discovered that IgM is essential for viral clearance whereas IgG does not appear until 7 dpi, when viral titers are already low (42). This model validation remains the only one to date that is based on such a large data set. The model itself is important because it breaks up the adaptive response into the roles of specific effector cells. In the case of comparing subtypes in order to examine pathogenicity, this differentiation is important because it quantifies differences in cellular responses. It is yet another way to identify differences between strains and more closer to understanding which differences result in more or less pathogenic qualities.

In a more specific look at the innate immune responses that directly explores virulence in a mathematical model, Smith, Adler et al examined the effect of the 1918 PB1-F2 protein on virus dynamics. They used data obtained from infecting mice with a virus that had either a wild-type PR8 protein or a genetically engineered PR8-PB2-F1 (1918) protein. They fitted a mathematical model to the data and found that mice with the 1918 protein exhibited a higher viral production rate and a higher infected cell death rate. They also saw inflammatory changes in the lungs with the addition of the 1918 protein,

possibly indicating that innate immune responses also play a role in the increased virulence of the PB1-F2 mutant (44). The significance of this model lies in the fact that they were able to quantify and suggest exactly why the 1918 PB1-F2 protein is more virulent. The results, that it causes a higher cell death rate and higher viral reproduction rate, have been reported in the previous studies (113,114), but it is really the process that is significant here. Using a model to identify the causes of increased virulence is a significant area of research that could be very useful in advancing our knowledge of what makes certain strains more virulent.

Pan and Hancioglu et al also looked at the effects of preexisting immunity and different serotypes on disease progression (58, 79) . Hancioglu et al used a separate variable to simulate antigenic distance of the invading virus from that of the host antibodies. For hosts with no preexisting immunity, disease was severe and the patient remained contagious for a longer period of time, increasing the probability of spreading the disease in a pandemic situation. They determined that a combination of host susceptibility and virulence determines the outcome of infection (79). Pan assumed that the contribution of CTLs and innate immune effectors was constant throughout infection because they can effectively recognize and attack new strains as well as previous strains. Antibodies, however, have decreased affinity to new serotypes that further decreases with increasing antigenic distance between the new and old strains. Pan separated B cells into two populations – naïve and memory – with different affinities. He found that competition between the two populations of B cells compromises the immune response. Native antibodies take time to mature and prevent memory antibodies from neutralizing the antigen. This suboptimal response was the worst for antigens with intermediate antigenic distance to an antigen recognized by the host immune system. The population of B cells that recognized the virus subtype secreted antibodies to neutralize it, while at the same time, naïve B cells that did not recognize the virus particle interfered with the neutralizing antibodies through their attempts to affinity mature. The two populations were competing for access to the antigen, allowing neither to do its job properly. In contrast, if the virus particle is new, the naïve population can develop an affinity for that subtype without hindrance. If the virus is recognized, it can be effectively neutralized (58). These two models suggest that antigenic distance of the virus plays a significant role in infectiousness, disease severity, and disease duration. For a new virus with virulent pandemic potential, this is an especially significant modeling area that should be explored further. It could

help quantify the effects of antigenic distance and is therefore provide another point of differentiation between subtypes. Further information on the significance of animal origin and subsequent human adaption would significantly aid in increasing our understanding of how viral origin plays a role in transmission and pathogenicity.

3.4.1 Modeling Antiviral Effects

Work done in modeling antiviral treatment of influenza A has called attention to the virus' ability to rapidly induce drug resistance. Beauchemin et al used an in vitro hollow-fiber system to model the effects of amantadine, a drug that blocks the abilities of the M2 protein. They showed that the drug only had a maximum efficiency of 56-74% depending on which model was used and demonstrated that this was due to the emergence of drug resistance (51). Using a model to explore the development of viral resistance to Amantadine might allow us to gain insight into the evolution of drug resistance and thus shape the development of new drugs. Model simulations performed by Lee et al emphasized the known fact that infection can be controlled if the antiviral agent is administered within two days of infection. Within this critical period, the drug limits viral load and prevents viral spread. They also found that drug combinations were more effective than one drug alone (48). Smith et al further defined this initial window of opportunity as Phase 1 in their two-phase model. Phase 1 represents the initial burst of viral growth that varies between patients, thus suggesting a patient dependent length of time for drug administration that usually hovers around two days (40).

Antiviral treatment at the beginning of infection also serves to minimize the development of drug resistant strains according to Handel et al. But once a resistant strain has developed, it has a high probability of spreading between hosts if the viral transmission capabilities remain unaffected (55). The mechanism of drug resistance was assessed by Holder et al. They observed that oseltamivir resistant H1N1 exhibited increased infectivity that compensated for an impaired viral release. The virus was slow in releasing progeny after infecting a cell but infected cells very quickly. This study was unique in that they did not attempt to fit a full model to their experimental data, but rather only considered the dependence of two quantities (viral titer and plaque velocity) on the parameters. This approach could also be useful in characterizing emerging strains or measuring the activity of new antivirals (56).

All of these studies emphasize the crucial approximately two day time period immediately after infection. With such a small window of opportunity, it seems that early detection and treatment is the best strategy, although such rapid diagnosis and drug administration would be incredibly challenging.

Table 3: Summary of Models for Potential Use in a Pandemic Model

Model Type	Authors	Use in Pandemic Modeling	Reference
Target-Cell Limited	Baccam et al	Basic model to be used as a foundation for increasing complexity.	39
	Smith et al	Suggests how viral infectivity, pathogenicity, and transmission capabilities can be compared between strains.	40
Incorporating Innate Responses	Saenz et al	Demonstrates a way to model high cytokine concentration and low cytokine effectiveness.	41
Incorporating Adaptive Reponses	Lee et al, Miao et al	Demonstrates differences between the responses of specific effector cells, which could be important when looking for differences between viral strains.	48, 42
Incorporating Additional Parameters	Smith, Adler et al	Shows how a model can be used to identify the causes of increased virulence and how it can be used to compare strains of differing virulence.	44
	Pan et al.	Provides a good method of modeling antigenic distance.	48

Table 4: Summary of Mathematical Models

Model Type	Authors	Summary/Unique features	Reference
Target-Cell Limited	Baccam et al	Basic model to be used as a foundation for increasing complexity	39
	Smith et al.	Separated the infection process into two phases defined by a change in viral growth rate.	40
Incorporating Innate Response	Baccam et al	Introduced equation modeling 1FN population.	39
	Saenz et al	Introduced equations modeling 1FN population, prerefractory cells, and refractory cells. Used equations to simulate the effects of IFN effectiveness and high 1FN load on disease severity.	41
Incorporating Adaptive Reponses	Bocharov and Romanyuka	Proved that innate and adaptive responses are essential for complete viral clearance.	80
	Hancioglu et al	Discovered that antibodies play a major role in viral clearance.	79
	Handel et al	Introduced an equation modeling the adaptive response	43
	Pawelek et al	Modeled the adaptive response by changing cell death rate at a specific time point	45
	Chang et al	Focused on CTL dynamics	49
	Tridane et al	Focused on CTL dynamics	78
	Lee et al	Separately modeled dendritic cells, CD4 T-cells, CD8 T-cells, and both IgG and IgM. Discovered that APCs significantly impact disease duration.	48
	Miao et al	Validated the model by Lee et al. Discovered that IgM plays a more significant role than IgG.	42
Incorporating Additional Parameters	Smith, Adler, et al.	A comparison of the effects of the PB1-F2 protein from a seasonal and a virulent strain on other parameters.	44
	Pan et al.	Separated antibodies into two populations, naïve and memory, and shows how the immune response is impacted by antigenic distance.	48
	Hancioglu et al	Used a separate variable to model antigenic distance and showed the impact it has on disease duration.	79
	Beauchemin et al	Modeled the progression of amantadine resistance.	58
	Handel et al	Modeled the development of drug resistant strains and the probability of the resistant strain spreading.	43
	Holder et al	Modeled the mechanism behind drug resistance	56
	Smith et al	Identified the window to administer antiviral drugs as Phase 1 in their two phase model.	40
	Lee et al	Indicated a critical period of for the administration of antivirals at the beginning of infection.	48

Chapter 4: Discussion

4.1 Future Directions for Current Models

In order to create a mathematical model, it is necessary to reduce all of the factors involved to only those that are relevant. But in the case of influenza A, relevant factors differ between serotypes. Herein lies the challenge of creating a model that accurately simulates the kinetics of influenza A infection. Different strains behave in different ways and, after all, it is the differences that are significant. Their variations and mutations are what give rise to increased or decreased virulence and transmissibility (14). A model generated based on data for seasonal H1N1 is not entirely representative of highly pathogenic H5N1 because it fails to incorporate parameters that are essential to that particular serotype. Hancioglu et al attempted to model differential virulence through changes in the rate of infection and the rate of viral particle secretion (79), but such an approach is not sufficient. There are many factors that affect viral titer and still more that contribute to virulence other than viral replication rates. In order to create a model that accurately captures a highly pathogenic subtype, many elements need to be considered. Some are included in models reviewed in the previous section and some have yet to be incorporated into models.

As previously discussed, avian viruses bind to SAα(2,3) receptors in the lower respiratory tract while humanized viruses bind to SAα(2,6) receptors in the upper respiratory tract. Preferential binding targets have an effect on replication rate, infectiousness, and virulence. The target cell limited model first used by Baccam et al upon which many subsequent modeling efforts have been based was analyzed with data from seasonal H1N1 infection which mainly infects the upper respiratory tract because it is a virus of swine origin. Viruses that infect the lower respiratory tract induce more severe disease and are more likely to lead to complications like pneumonia (89). Viruses with this specificity also induce higher levels of proinflammatory cytokines in macrophages and epithelial cells (88). Both of these results were independent of viral replication. In short, they behave in a different manner from human adapted viruses that infect the upper respiratory tract. Therefore, it is a disservice to only include the characteristics of upper respiratory tract infection and to model virulence solely through replication rates. The preferential binding receptor of the virus in question should be taken into account. This could be done by compartmentalizing the model into two sections – the upper and lower respiratory tract – and approaching the influenza infection as separate populations. A dual specificity was observed with the 2009 H1N1 virus

(96), so this approach might be useful. It could potentially highlight important kinetic differences between viruses with SAα(2,3) and SAα(2,6) receptor specificity. A possibly less complicated alternative would be to introduce an equation modeling the cytokine population as a whole in order to capture the increase in cytokine production during lower respiratory tract infection. Excessive cytokines, as explained previously, are damaging to the host and so an equation that models their dynamics would account for the changes in viral behavior associated with a different receptor specificity. However, this approach might be limiting as all of the consequences of a SAα(2,3) specificity are not known. There might be other host repercussions beyond replication rate, cytokine release, and host range when considering SA preference.

The ability of highly pathogenic strains to prevent premature apoptosis is also a unique and important feature that should be included in existing models. The rate at which infected cells I_2 die, represented by δ in the model by Baccam et al (39), would be slower than that of a less pathogenic virus. With highly pathogenic viruses, the death rate of infected cells is not dependent solely on the number of cells infected. It also depends on the ability of the virus to delay apoptosis, which differs between strains and is higher for some viruses with increased virulence. A solution to this problem is introducing a new variable that compensates for decreased infectious cell death. This variable could be included in the equation modeling the rate of change of the infected, virus-producing cell population in the target cell limited model and could represent the amount of TGF-β produced in the host because it has been shown that TGF-β plays a role in apoptosis prevention (60). In this way, TGF-β would serve as a proxy for the ability of a given virus to prevent premature apoptosis. IFN is used in models in exactly this manner. The amount of IFN measured in the host indicates the strength of the innate immune system. The amount of TGF-β produced would similarly represent the success of the virus in preventing host cell apoptosis. The target cell limited model is inaccurate in assuming that the death rate of infected cells is dependent solely on the number of infected cells present. A slower death rate would mean higher, more sustained viral replication, which has been observed with both H5N1 and 1918 H1N1 (92). Including the prevention of apoptosis as either a separate variable or as a modification to the parameter δ would improve the ability of these models to accurately model the kinetics of serotypes with virulent pandemic potential.

As mentioned above, IFN production is an important variable in influenza A models because it serves as a proxy for the entire innate immune system. The amount of IFN produced by the host indicates

the level of host immune response to the virus as well as the level of protection afforded to uninfected cells. However, there are two problems with this approach when considering highly pathogenic viruses. The first of these is the issue of IFN representing the entire innate immune system. It is used because it recruits the effectors of the innate immune system - NK cells and macrophages - and it primes uninfected cells against infection so it essentially has been used to represent all aspects of the innate response (96). It is also relatively straightforwardly measured in animal models, adding to ease of experimentation. However, IFN cannot be used to represent the entire innate immune system because influenza A infects the macrophages and NK cells that are recruited by IFN. The virus has been shown to replicate within both cell types and inactivate NK cells (5, 6). So if innate immune activity is fully represented by IFN, the detrimental effects of influenza A virus to effector cells is not accounted for. IFN recruits the cells, but they may or may not have an effect on viral removal. It is likely that the level of effector inactivation is serotype dependent. This is an area that should be explored in further studies and could be represented in the existing equation simply by a reduction of the IFN population, representing the shortcomings of the innate immune system due to viral evasion techniques. Also, by looking at more variables of the innate response independently, the modeler would be able to demonstrate the role and relative importance of each effector on the response.

Additionally, the PB1-F2 and NS1 proteins of more virulent strains down regulate IFN production (21, 24, 29). Therefore, it is important to consider the extent of IFN down regulation and its effect on the host response to infection. Due to the ability of these serotypes to decrease the amount of IFN in circulation, the response of effector cells is altered as is the protective response of uninfected epithelial cells. Saenz et al addressed this viral feature by using the changes in refractory cell population to model IFN effectiveness (41). Smith et al looked at the effect of the PB1-F2 protein on viral reproduction but didn't create a separate variable (44). Smith et al observed the effects on existing model parameters instead, measuring viral titers and reproduction rates. It would be informative to perform a series of experiments that determined exactly how much IFN production decreases due to the mutation in PB1-F2 and a separate experiment that determines the same for the mutation in the NS1 protein. Looking at the impacts of mutations in these two proteins on viral kinetics, as Smith et al did for PB1-F2, would provide interesting insights and useful quantitative measurements. The model parameter developed by Saenz et al is a productive step towards effectively modeling pathogenic serotypes but further development is needed.

It requires additional validation through more experiments to demonstrate that it is an accurate way to represent the down regulation of IFN.

Hypercytokinemia is likely the most underrepresented viral characteristic in influenza A modeling. Cytokine production is a key determinant of viral pathogenicity. Over production of cytokines has far reaching effects, including cell damage and excessive inflammation (81). This lethal characteristic is not present in less virulent strains and therefore is not included in existing influenza A models. Saenz et al discovered that high levels of IFN were detrimental to the cell but did not expand the model to include other cytokines because they were only looking at the decrease in innate immune response (44). Many cytokines that are released during infection are proinflammatory and perform functions that allow immune effectors to reach the site of infection and perform their job, but if they overreact in the case of a cytokine storm, the damage is rapid and severe (31). The potential for hypercytokinemia is not taken into account in current mathematical models. In the case of H5N1 and 1918 H1N1, the disease severity is directly correlated with over amplification of cytokines due to high replication rates (81-84), indicating that levels of cytokine production is a very important feature in these infections. To fully capture the effects of cytokine storm, the cell damage would have to be taken into account as well as the high density of immune effectors that are recruited to the site of infection. Cell and tissue damage by cytokines affects the model by a change in cell death rate and an increase in mortality rate of the host. Cytokines at high concentration damage surrounding tissue as well as cells susceptible to influenza A infection, leading to increased host mortality. An equation that models the cytokine population would likely capture this feature. It would clearly show the impact of an increase of cytokines on the mortality rate on disease severity.

Similarly, the cleavability of the HA protein has been shown to be an essential parameter in differentiating between viruses of varying pathogenicities. It is likely not included in any influenza A mathematical models because most models to date are formulated using data from less virulent strains. If HA cleavability were included as a parameter, it would not only serve as an indicator of viral replication rates and virulence, but it would also represent the likelihood that the virus would establish a systemic infection. Models are currently focused on modeling the kinetics of the upper respiratory tract and ignore the viral effects on the rest of the body that take a toll on the host. Systemic infection has been implicated in lethal cases of influenza A and is a likely cause of patient deterioration (85, 87). Ignoring the HA

cleavability would mean discounting the effects of the virus on the rest of the body. A way to include this as a factor would be the addition of a separate equation that models the change in blood viral titer over time. The modeler would likely find a correlation between the onset of severe symptoms and time at which the virus enters the blood stream. Data for this parameter could be collected using animal models or published experimental data of H5N1 victims.

All of modifications and additions to current models that are discussed here should be viewed as equations to incorporate into quantitative models that reflect highly pathogenic influenza A viruses. Introducing additional parameters, such as IFN effectiveness or cytokine level, would allow for greater model flexibility and would enable the user to model differences in serotypes. It would facilitate the creation of a single model that represents influenza A as a whole and the modeler would simply need to adjust parameters in order to model a different strain. A model such as this could be used to identify and characterize strains with virulent pandemic potential if certain additional parameters were also added. The next section discusses what such a model might include and how it would be beneficial.

4.2 Necessary Components of a Model That Assesses Pandemic Potential

Influenza A models developed thus far have mostly been based on less virulent viral strains and have differed in which aspect of viral infection they represent. Some tackle only the innate immune responses while others include both the innate and adaptive immune system and still others look at an entirely different area such as cross-productivity, but none so far attempt to model the whole picture. A model that combines all of these different features would provide additional insights into the kinetics of influenza A and, when combined with the transmittance capabilities of the virus, would serve as the template of a predictive model that would capture the virulent pandemic potential of a particular virus. The three essential components of a virulent pandemic influenza model are: virulence, transmission, and cross-productivity. If all three of these factors separately fall under the category of potentially virulent pandemic risk according to pre-established criteria, then the virus as a whole should be flagged as a risk and appropriate measures should be taken. The way in which one would assess whether the criteria is met for each of these is discussed below.

The Three Necessary Components of a Model That Assesses Pandemic Potential:
• Virulence • Transmission • Cross-protectivity

Virulence would be an attribute that encompasses infected cell death rate, viral rate of replication, susceptible cell infection rate, and cytokine production. The elements discussed in the previous section such as apoptosis prevention and viral evasion of host the host immune system are factors that influence overall virulence. A method that could be used to define whether or not a virus is considered to be highly virulent is comparing the virulence of many viral strains from seasonal H1N1 to highly pathogenic H5N1 using the model and developing a set of parameters that function as threshold points. For example, a virus that has a replication rate and induces a host cell death rate above the threshold would qualify as virulent by this measure while another would not. All of the factors previously discussed which contribute to replication rate and host cell death rate, such as HA cleavability and preferential SA binding, would be incorporated in the mathematical model and would therefore impact the output values. The ultimate result of all of these measured values would be a value defining the virulence of the strain in question. Modeling could establish a quantitative method for predicting virulence among influenza A subtypes.

The ability of a virus to transmit between people is an essential parameter in defining its virulent pandemic potential and can be included simply as a binary value. The virus can either be passed between people or it cannot, and its ability to do so is determined by its binding preference to either SAα(2,6) or SAα(2,3) and the G627K mutation. The effects of binding preference and the mutation on virulence (increased replication rate, cytokine release, etc.) should be handled under the 'virulent' component of the model. This parameter is more black and white than virulence and should simply indicate whether or not a subtype is capable of spreading between people.

A strain that poses a threat for pandemic spread will be a new serotype for which the human population has no or limited cross-productivity, so a feature that looks at antigenic distance is important. The impact of a new strain is determined in part by how "new' it is, or how easily our immune system can recognize and eliminate it (97). Hancioglu et al proposed a variable for the representation of cross-

protectivity that could be incorporated to represent this feature. They defined antigenic distance as a difference in compatibility between the virus and the antibodies produced by the organism (79). An alternative way of measuring this feature would be to compare the sequence of the new strain against known circulating seasonal strains of the same HA subtype because, due to its role in the infection process, it is ultimately the HA that determines the extent of cross productivity (99). Measuring the percent homology between the new virus and viruses with the same HA subtype would provide an accurate input to for the model. This parameter could easily be measured by doing a BLAST protein search with the protein sequence.

The ultimate model would be one that includes all three components and operates based on the viral sequence. With this model, a person plugs in the genotype that includes phenotype-altering mutations and can then view the differences in pathogenicity and transmittance between strains. Virulence, transmittance, and antigenic distance would be defined as stated so that the model output would be a three variable reading. All three variables are necessary to the model and all should have a specific definition of what qualifies them as potentially virulent pandemic independently. Although 2009 H1N1 is easily spread between people, it did not cause severe virulence because of its low virulence and preexisting immune status among the population (98). Yet it was declared as a pandemic in 2009, a decision that was subsequently met with criticism. With the model discussed in this paper, virulent pandemic potential could be projected and precautions would be taken depending on the model output. Viral sequencing is easily performed and is continually evolving and improving. If one obtained the sequence of a new virus and then, based on the mutations it contained, inputted the information into the mathematical model, the output would indicate its degree of risk and may project useful descriptive values such as peak viral titer, cytokine concentration, and host cell death rate. From these values one could answer questions such as: Is the virus exceptionally virulent like H5N1? Does it transmit readily like 2009 H1N1? Or does it display both characteristics like 1918 H1N1? All of these questions could be explained without performing lengthy and costly laboratory experiments. We are a long way from developing the ultimate model with the current state of knowledge and technology, but given the rapid rate of growth of influenza A modeling in recent decades, a model useful for projecting virulent pandemic potential could someday become a reality.

4.3 Challenges to Mathematical Modeling

In many ways, the construction of the proposed model is dependent on advances in modeling and influenza A research. It is still not understood why when H5N1 switched receptor binding specificities from SAα(2,3) to SAα(2,6) it lost much of its virulence while 1918 H1N1 did not (1). It also remains to be understood if TGF-α plays a greater role than just the prevention of apoptosis in epithelial cells (62). Also important to the model proposed would be further discoveries of which specific mutations in the viral genome affect the phenotype. Some are definitively understood, such as the presence of arginine in the HA binding pocket or the amino acid switch from lysine to glutamate at position 627 in the PB2 protein. Others, like the sequence(s) that determine HA cleavability, are unclear. A polybasic cleavage site is essential for H5N1 lethal infection, but the 1918 H1N1 does not have a polybasic cleavage site yet still exhibits high mortality rates (100). Additional insights into the biology of the influenza A virus and the characteristics that differentiate pathogenic strains from seasonal strains would be hugely beneficial to model development.

Finding a balance for model specificity is also a challenge. Over simplification of model parameters does not provide any useful insight, while over complication of the model defeats the purpose of modeling. Identifying the relative importance of model parameters will help to solve this problem. Some have a significant impact on viral kinetics, while others do not.

More model validation is also increasingly important. Only one study performed by Miao et al used a sufficiently large data set of 340 mice to generate the data necessary to fine tune their model parameters. Baccam et al fitted only 6 sets of data to their model and many subsequent models are based on this template. Studies such as these are certainly challenging but will be increasingly important to conduct as models continue to grow in complexity.

4.4 Public Health Benefits of a Model that Assesses Pandemic Potential

A major benefit of the creation of a model that predicts virulent pandemic potential would be alleviating needless public crises like the 2009 H1N1 outbreak and the recent media frenzy surrounding H5N1 laboratory generated mutants. If a model was available to analyze these serotypes, there would be more information surrounding their virulence or their transmission capabilities. The United States spent $4.17

billion during the H1N1 outbreak, mostly on vaccines and on grants distributed to the states (101). The actual pandemic was not as severe as was predicted, so it is likely that money could have been saved if the government had a reliable predictive tool. Conversely, in the event of the emergence of a highly pathogenic strain resembling the 1918 H1N1 virus, the government and citizens would hopefully be able to take appropriate measures to ensure that the mortality rate is significantly lower. Experts state that an influenza A pandemic is inevitable and possibly imminent (102). All we can do is limit its impact through preventative measures.

Additionally, incorporating comprehensive within-host influenza A model into an epidemiological model would provide additional information on its global spread. This has already been done to some extent in models exploring areas such as the impact of seasonality and viral mutations on transmission (103) and in models looking at the influence of prior immunity on pandemic severity and spread (104, 105). HIV modeling, which has progressed by leaps and bounds in recent years, has gained new insights due to the merger of these two disciplines (106). Other more general models based on infectious disease have been developed with the goal of successfully unifying within and between host models (107,108).

Treatment strategies could also be effectively analyzed through a comprehensive model. Several modelers have already shown that combinatorial drugs are more successful than a drug by itself (40,48) and others modeled the progression and spread of antiviral resistance (51,55). Knowing the susceptibility of an emergent strain to antivirals and its likelihood to develop resistance would be an important tool in stockpiling treatments during pandemic preparedness. The applications in the pharmaceutical world are important as well. Monoclonal antibody treatments have been a major focus in the biotechnology industry in recent decades and could be effectively analyzed through an all-inclusive model. One could potentially measure the necessary dose to bind an effective portion of viral particles or neutralize cytokines in the event of a cytokine storm. Quantitative information on the cytokine population and viral titer could be generated from the model that could then be used to estimate impact and dosing.

The benefits that could result from the development of a comprehensive model that predicts pandemic potential are significant. Although recent advances have been substantial, there are many more questions to be answered and many problems that have yet to be solved. By making the modifications and additions that this paper suggests, current models will better represent highly pathogenic strains.

Additionally, complementary efforts in modeling and the generation of experimental data, preferably in tandem, will aid in enhancing our understanding of infection dynamics of influenza A and will provide the data necessary to improve current models. If modeling efforts continue to move forward with the momentum of the last two decades, it is likely that we can expect further contributions towards the understanding of influenza A infection and progress towards the eventual creation of the ultimate model.

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