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Evaluation of Antibodies Induced After Vaccination with H1N1 Vaccine Candidates Created by DNA Shuffling

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Evaluation of Antibodies Induced After Vaccination with H1N1 Vaccine Candidates

Created by DNA Shuffling

Baylor DeVries

University of South Dakota

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Chapter One

Introduction

<u>General features of Influenza viruses and infections</u>:

Nearly 700,000 people are hospitalized annually due to influenza virus infections in the United States alone, with outbreaks causing between 12,000 and 56,000 deaths every year (Maggie Fox, 2018). These numbers do not always include those who die from other complications associated with the virus such as diabetes and cardiovascular disease. The virus is spread in small droplets via cough, sneeze, or touch, which can infect individuals (Influenza (Flu), 2018). First or second hand contraction can occur by someone sneezing, causing the droplets to enter your mouth/nose (Influenza (Flu), 2018). Additionally, the virus can exist on contaminated surfaces therefore leading to possible infection if touched. Once the virus enters your body, immediate action must be taken by your immune system to defend its host. The immune system responds by sending white blood cells, such as T cells and other inflammatory molecules such as cytokines, to attack the virus (Jabr, 2017). The main part of the body where the virus resides is in the lungs/respiratory tract. If the immune system responds properly, the virus should be cleared from the host and recovery takes approximately one week. If the immune system responds too aggressively, the inflammatory molecules produced can destroy too much lung tissue, causing the lungs to be incapable of delivering the proper amount of oxygen to the blood, leading to death (Jabr, 2017). Other secondary complications such as bacterial pneumonia, encephalitis, and myocarditis are very dangerous as well. This is why the development of a universal flu shot is critical, with its ability to save millions of lives.

The CDC categorized influenza into five different categories: seasonal, avian, swine, variant, and pandemic (Influenza (Flu), 2018). It is also broken down into 4 different virus types: A, B, C and D. Due to the different categories and viruses circulating at any given time, the vaccine is constantly changing each year. Scientists and researchers review and study the vaccine composition annually hoping to protect as many people as possible. The CDC, the FDA, physicians, pharmacists, and nearly all other experts agree that the most effective way to combat this disease is through the influenza vaccine. In Dr. Huber's laboratory I was able to test the ability of a universal influenza vaccine candidate to induce antibodies against both human and swine viruses using hemagglutination inhibition assays.

<u>Vaccine-induced immunity against influenza viruses</u>:

Hemagglutination inhibition assays (HAI assays) are used to detect and quantify the concentration of antibody in serum and rely on the natural ability of influenza viruses to bind red blood cells through a process known as hemagglutination. The HAI assay is the gold standard for antibody detection against these viruses. HAI assays are used to show if an influenza virus causes hemagglutination in blood, or if antibodies present in a sample can inhibit hemagglutination. Influenza viruses are composed of a glycoprotein envelope that can bind to sialic acid molecules found on the sialic acid residues that serve as receptor sites on red blood cells (Racaniello, 2009). Usually when red blood cells are put into a saline solution the cells will gradually drop to the bottom of a tube due to gravitational forces, appearing as a red dot. However, when viruses are added to the solution, interactions between the virus and red blood cells may be seen. The red blood cells are prevented from falling to the bottom on the tube and instead a shield is

formed keeping the cells suspended (Racaniello, 2009). This can be seen as a foggy red liquid rather than a single red dot (**Figure 1**). If a patient's serum contains antibodies that block viral attachment to red blood cells, this interaction is inhibited, and the red blood cells will settle to the bottom of the tube in a manner that looks like virus is not present. Influenza viruses are highly specific to the antibodies used within HAI, making the use of this method highly effective for antibody detection. The assay can be easily modified to determine the level of antibodies against influenza virus that are present in serum samples, with pig and mouse serum being among some of the most popular sera samples to test for antibodies after vaccination. In this study, I used the HAI assay to test specific influenza viruses against sera taken from animals that were vaccinated with an H1N1 universal vaccine candidate.

Every influenza season is different, with variants of the influenza A and B types, notably represented by the influenza A subtypes H1N1 and H3N2, circulating every season. This makes matching the vaccine with the circulating variant nearly impossible. The traditional trivalent vaccine (annual vaccine) protects against H1N1 (Influenza A), H3N2 (Influenza A), and influenza B virus, while the quadrivalent vaccine includes an additional influenza B virus (CDC, 2018). In 2009 we saw a novel variant of the H1N1 virus spread around humans causing a global pandemic. This virus contained swine, human, and avian genetic components, and was vastly different from the variants that circled during the past 30 years. This meant that the novel virus and the vaccine did not match, leading to more incidences of influenza, which coincided with increases in hospitalization rates and deaths. Since the immune system of children are naïve, and the immune systems of elderly are weak, both populations are more vulnerable to

severe influenza virus infections and are at a higher susceptibility to death (Jabr, 2017).

However, despite these hospitalization rates, in 2009, young adults were the group that

was most affected by this novel H1N1 virus that replaced the seasonal H1N1 variant

that was previously circulating (Jabr, 2017).   A seasonal vaccine against this novel

variant still remains the gold standard of protection against influenza. Since matching

each variant on an annual basis is also very time consuming (Gerdil C., 2003), broad

immunity may be a possibility in the future. However, the annual changes that occur as

influenza escapes host immunity makes induction of broad immunity very difficult. In

order to increase the breadth of immunity induced, a DNA shuffling strategy was

proposed as a potential solution.

Can we create a vaccine that induced immunity against multiple H1N1 variants?:

DNA shuffling is a process of randomly recombining parental genes to create a

novel gene that expresses a protein containing desired properties of the individual

parental genes (Joern, J. M., n.d.). In our case, DNA shuffling is being used to create a

single protein that can induce immunity against 4-5 parental influenza A H1N1 variants.

This is very important for influenza viruses because they can cross a species barrier,

which was the case in 2009. The novel H1N1 variant, also known as the swine flu, had

the ability to move freely between pigs and humans (Berlanda Scorza, F., et al., 2016).

The method used in the Huber lab induces immunity against the HA protein using

epitopes, which are the part of an antigen molecule to which an antibody attaches itself.

This method is intended to both prevent transmission between pigs and humans, and

stop virus spread among pigs and among humans. The approach could be utilized to

create a pre-pandemic vaccine that would also be useful during the early stages of a

pandemic. If we can prevent the virus from circulating within the swine population, this may ultimately mean a decrease in transmission from pigs to humans as well.

Previous work in the Huber lab showed that recombined chimeric genes created by DNA shuffling can attain the properties described above. Specifically, a single protein had the ability to induce antibodies against 4-5 parental variants (McCormick, et al., 2015). These "desired genes" specifically contain influenza A virus hemagglutinin (HA) sequences and the ability to induce antibodies was evaluated in both mouse and pig models (McCormick, K., et al. 2015). Molecular breeding can speed up the evolution of a virus (Crameri A, Raillard, 1998), and this was the method used to create the previous vaccine against influenza A in pigs. One restriction of the vaccine produced was that only one of the HA's could be expressed within a conventional vaccine, and this HA was known as HA-129. However, screens that were done using DNA vaccines for all of the chimeric HAs produced showed that one additional gene-shuffled products, HA-113, could induce immunity with increased breadth and strength, compared to HA-129. Although we were unable to express HA-113 using conventional vaccine methods that were available in the Huber lab, we were able to utilize the parainfluenza virus 5 (PIV-5) technology that was available through collaboration with Biao He (University of Georgia, Athens, GA). PIV-5 was used as a vector for delivery of influenza hemagglutinins created using DNA shuffling as universal vaccine candidates (**Figure 2**), specifically the HA-111 and HA-113 genes.

Parainfluenza virus-5 (PIV-5) as a vaccine vector:

Many viruses can enter though the respiratory tract. This includes the influenza virus, paramyxoviruses, coronaviruses, pox viruses and herpes viruses (Clark, K. M., et

al. 2011). Paramyxoviruses can infect the respiratory tract and cause a wide variety of human and animal diseases (Welch BD, et al., 2013). We have chosen PIV5 as a vector for vaccine delivery based on a number of characteristics of this paramyxovirus. First, the vaccine has been demonstrated as safe as it was tested in a live vaccine against kennel cough in dogs. No adverse events were reported. Additionally, PIV5-based vaccines will not interfere with prior immunity against PIV5 (Chen Z., 2012). Lastly, PIV5 vectors can induce immunity against different influenza A virus subtypes in mice, thus PIV-5 is a viral vector that is a promising candidate for vaccine development (Li Z., et al., 2013). With this information, PIV5 was utilized as our vector for influenza HAs, and here we report on the testing of a PIV5 expressing HA-113 (PIV5-113) in both mice and pigs. This PIV5-113 vaccine induced broad immunity in both animal models, with quantifiable readouts being detection of antibodies and the ability to protect against virus infection. The findings are presented below and discussed in the context of how broad vaccine-induced immune responses can prevent both seasonal and, potentially, pandemic influenza virus infections.

Chapter Two

Methods and Materials

Parental HA Genes

The HA genes of parental H1N1 influenza A viruses, including A/Tennessee/1-560/09 (TN09), A/NewJersey/8/1976 (NJ76), A/Ohio/01/2007 (OH07), and A/Iowa/01/2006 (IA06) were isolated from stock viruses and cloned as described (McCormick, et al., 2015). Additional H1N1 influenza A virus strains, were used to test antibodies induced for cross reactivity, as described below. Viruses expressing TN09, NJ76, OH07, and IA06 HA proteins were used to determine the reactivity of HA-113-induced antibodies against the parental viruses. The primary viruses expressing HA proteins from the A/Germany/81, A/Iowa/92, A/Brisbane/07, A/California/09, A/Michigan/15, and A/swine/Iowa/13E100/2013 were used to test the breadth of immunity induced by the vaccine candidate. A/Brisbane/59/2007 HA is antigenically similar to the A/Memphis/3/2008 HA, and both of these viruses circulated in humans immediately prior to the H1N1 pandemic in 2009. Additionally, the A/Michigan/2015 virus represents the H1N1 variant that was included in the 2017 human vaccine, replacing the A/California/4/2009 isolate that had been in the vaccine since 2009. The A/swine/Iowa/13E100/2013 virus was isolated from a nasal swab taken from a healthy pig in Iowa in May of 2013. The A/Michigan/2015 virus and the A/swine/Iowa/13E100/2013 virus are particularly crucial in the evaluation of our PIV5-113 vaccine because these viruses were both isolated after the 2009 pandemic. Since 2009 was the last year for inclusion of a parental HA in the vaccine, these viruses are being used to determine whether the vaccine can induce immunity against future human

(Michigan) and swine (13E100) viruses that circulated in their populations after the vaccine had been created by DNA shuffling.

Creation of PIV-113

Using sequences from previously shuffled genes (McCormick, et al., 2015). Biao He created PIV5 vaccine expressing HA-113 (PIV5-113). This was done by expressing the DNA sequence from the shuffled HA gene HA-113 (McCormick, et al., 2015) in PIV5 (ZL48 virus) between the genes expressing hemagglutinin-neuraminidase (HN) and the large RNA polymerase (L) proteins (Mooney AJ., 2013).

Vaccination using PIV5-113

Using PIV5 expressing HA-113 (PIV5-113) as a vaccine, post-vaccination sera samples were taken on days 7, 14, 21, and 28 and tests were performed to monitor antibody production. Mice were immunized with PIV5-113 and then given a booster on day 28 and sera were collected three weeks later. Pigs were immunized with PIV5-113 and given a booster 14 days later. After vaccination, pigs and mice were challenged with influenza viruses and nasal swabs were taken on day 1 post challenge, day 3 post challenge, and day 5 post challenge. Tests including the HAI assay, as described below, were performed. Focusing on HAI assays, the sera samples provided sufficient data to show antibody production against parental influenza strains. In this study we evaluated vaccine-induced immunity using PIV5 expressing HA-113 (PIV5-113) using both mouse and pig models of vaccine:challenge. As a control, the vector alone (PIV5 alone) was delivered to both mice and pigs.

Hemagglutination Inhibition Assay (HAI)

Hemagglutination inhibition (HAI) assays were performed to evaluate antibody levels in the sera collected (**Figure 1**). The highest dilution of serum that prevents hemagglutination is called the HAI titer for that serum sample (Acharya, 2018). To perform the HAI assay the virus is diluted across a 96-well, round-bottom plate (50µl PBS to columns 2-13, 100µl virus in the 1st column). A solution of 0.5% red blood cells in 50 microliters is then added to each well to determine the amount of virus present. After 30 minutes, hemagglutination can be observed. The virus stock is diluted to a 1:8 titer in 50 microliters and tested to confirm a titer of 1:8 using the dilution system described above. Once the virus stock is at a 1:8, it can be used in the HAI assay to determine whether the antibody present in the serum can inhibit virus interactions with red blood cells.

For the HAI assay, a round-bottom 96-well plate is used, and 25 microliters of PBS is added to each well with the exception of the first well in a row or column (depending on the dilution scheme). Mouse and pig sera that are used in the HAI assay are initially treated with RDE, heat inactivated, and pre-cleared with red blood cells to remove natural inhibitors to the assay, as described (McCormick, et al., 2015). After treatment, serum samples are added to the first well in a 50 microliter volume and diluted as described above for virus, using 25 microliters as the transfer volume between wells. After the sera have been diluted, 25 microliters of virus (4 hemagglutinating units, based on the 1:8 titer in 50 microliters that was determined previously) is added to each well. Plates containing diluted antibody and virus are incubated for an hour before adding 50 microliters of 0.5% red blood cells. After 30

minutes the HAI titer can be observed and recorded. Since antibodies against influenza HA have the ability to prevent attachment of the virus to the red blood cells, the highest dilution of serum that prevents hemagglutination (hemagglutination inhibition) is called the HAI titer of the serum (Racaniello, 2009). Titers are reported as the reciprocal of the final serum dilution that inhibits hemagglutination. A titer 5 is assigned to serum samples that did not demonstrate HAI at the starting dilution of 1:10. HAI assays were performed on both mouse and pig serum.

Mouse Challenge

Mice were immunized with PBS, PIV5, or PIV-113. They were given a second dose of their respective vaccine 28 days later as a boost, and sera were collected 20 days after this second inoculation. Mice were challenged with mouse-adapted viruses expressing the parental HA's from A/Tennessee/1-560/09 (TN09), A/NewJersey/8/1976 (NJ76), A/Ohio/01/2007 (OH07), and A/Iowa/01/2006 (IA06), and A/Memphis/3/2008 (ME08). These viruses were created as described previously (McCormick, et al., 2015), and mouse challenge studies were performed as described (Huber, 2009).

Pig Challenge

Pigs were immunized with PBS, PIV5, or PIV-113 and boosted with their respective vaccines on day 14. Sera were collected 14 days later and challenged with the viruses. Nasal swabs were taken on day 1 post challenge, day 3 post challenge, and day 5 post challenge. These viruses were created as described previously (McCormick, et al., 2015).

Ethics Statement

All work performed with influenza viruses was done in accordance with protocols that were approved by the Institutional Biosafety Committee of the Unviersity of South Dakota (USD) and Kansas State University (KSU). Work in mice was done following experimental conditions approved by the Institutional Animal Care and Use Committee (IACUC) of USD, while work in pigs followed IACUC-approved procedures established at KSU.

Chapter Three

Results

In this study, both mice and pigs were vaccinated with a Parainfluenza Virus (PIV-5) vector that expressed HA-113 (PIV5-113, **Figure 2**). I will first discuss the results found with the challenge in mice, which was work that was completed by Sarah Zaiser before I joined the Huber lab, and then I will discuss the results for the vaccine and challenge of pigs, which was done by Ying Fang at Kansas State University (Manhattan, KS). My specific role in this study was to use the HAI method to test and determine the antibodies induced after vaccination in both mice and pigs. The complete results are being presented as a way to relay the contribution of my work in the big picture of the project.

Mice vaccinated with PIV5-113 demonstrated immunity against individual influenza viruses that expressed HA proteins from all 5 parental viruses (**Figure 3**). All of the PIV5-113 vaccinated mice that were challenged with NJ76-, IA06-, OH07-, and TN09-expressing, mouse-adapted viruses showed protection against the lethal challenge, while none of the mice challenged with the ME08-expressing virus survived (**Figure 4**). This means that although antibodies were induced against ME08 by the PIV5-113 vaccine, the levels were not high enough to provide protective immunity. The gold standard for protective immunity against influenza infection is an HAI titer of 40, which is a titer that is defined for population studies. It is known that 50% of people that have a titer of 40 will be protected from virus infection, but individuals may vary in the relationship between antibody titer and protection against infection. ME08 had an
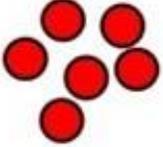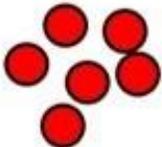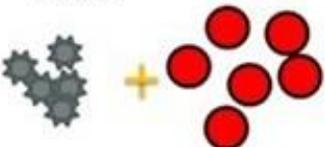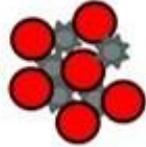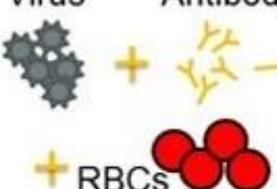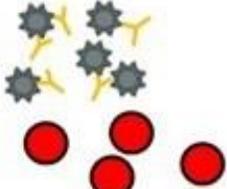
average titer of approximately 20, which was not strong enough to provide protection against this virus.

Since immunity was achieved in the mice using the novel PIV5-113 vaccine, a vaccine:challenge study was performed in pigs. Pigs are a more closely related species to humans and pandemics arise from swine, making them a vital tool in vaccine research and an important population to test our vaccine. In fact, the PIV5-113 vaccine has the ability to be mass-produced and used directly in pigs, if it can demonstrate value in pilot studies like this. Pig serum samples from PIV5-113-vaccinated animals were tested for their reactivity against viruses expressing NJ76, IA06, OH07, TN09 and ME08 HA proteins. Results show an antibody response against all viruses except ME08 once again (**Figures 5 and 6**), which is not surprising since immunity against this virus did not reach a titer of 40 in mice either. Similar to the results in mice, we would predict that challenge of pigs with viruses expressing NJ76, IA06, OH07, and TN09 would be protective. Meanwhile, a challenge with a virus expressing ME08 would not show protection since it had a titer of approximately 20. These results show that our PIV5-113 vaccine could induce immunity in pigs similarly to what was observed in mice.

To determine whether PIV5-113 could induce broad immunity in pigs, we performed HAI assays using non-parental viruses expressing HA proteins from the IA92, CA09, MI15, GE81, BR07, and 13E100 viruses (**Figure 7**). These viruses are from the H1N1 family, and many were used in our previous study to demonstrate the breadth of immunity induced in pigs with the HA-129 vaccine (McCormick, et al., 2015). These were chosen because they are viruses that circulate in both pigs and humans, including viruses that circulated after our vaccine was created. The results show that

viruses expressing the BR07 and GE81 HAs had HAI titers that were less than 40, similar to the ME08 virus. Once again this would predict an inability for our vaccine to protect against direct challenge with these viruses. However, HAI titers against CA09, which was the H1N1 isolate included in vaccines starting in 2009 and is closely related to TN09, were similar to those detected against NJ76, IA06, OH07, and TN09, predicting protection. Interestingly, the MI15 and 13E100 viruses that represent viruses circulating in humans in 2015 and pigs in 2013 reacted with antibodies induced by PIV5-113 vaccines, showing that the PIV5-113 vaccine could provide immunity against influenza viruses that circulated in pigs and humans after its creation in 2009. This shows an added value of our vaccine. It indicates that with updates, a higher titer may be able to be achieved against all of these viruses. Since both Cal09 and MI15 had titers over 40, the PIV5-113 vaccine has potential as a candidate vaccine for current and future vaccines. In the future we will continue to test viruses post 2009 pandemic to provide more information related to virus evolution away from the breadth of protection provided by PIV-113.

Legend

Figure 1: This figure shows hemagglutination versus hemagglutination inhibition as described in the methods and materials section (Acharya, 2014). Since antibodies against influenza HA have the ability to prevent attachment of the virus to the red blood cells, the highest dilution of serum that prevents hemagglutination (hemagglutination inhibition) is called the HAI titer of the serum (Racaniello, 2009).
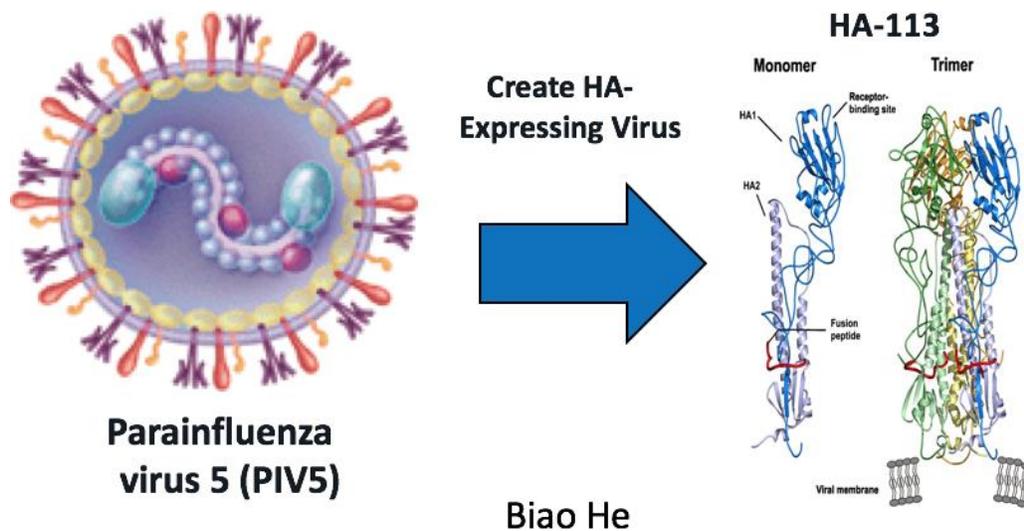
Figure 2: This figure shows how an HA expressing virus (novel HA) was created, PIV5-113. Biao He created PIV5 vaccine expressing HA-113 (PIV5-113) using previously shuffled genes. PIV5 was utilized as a vector as described in method and materials. Picture provided by Biao He.

Hemagglutination Inhibition After Vaccination Mouse Study
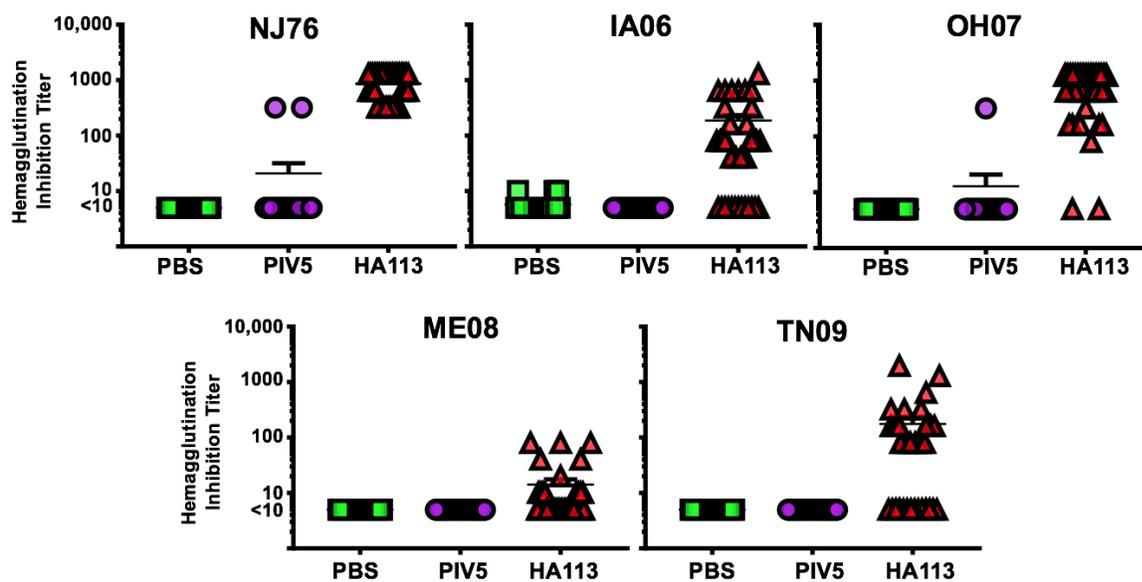
Figure 3: The data above shows the reactivity of sera from pigs vaccinated with HA-113. Reactivity against parental HAs is shown. The number of mice per vaccine group were as follows: 20 mice for PBS, 40 mice for PIV5, and 39 mice for PIV5-113. Data provided by Sarah Zaiser.
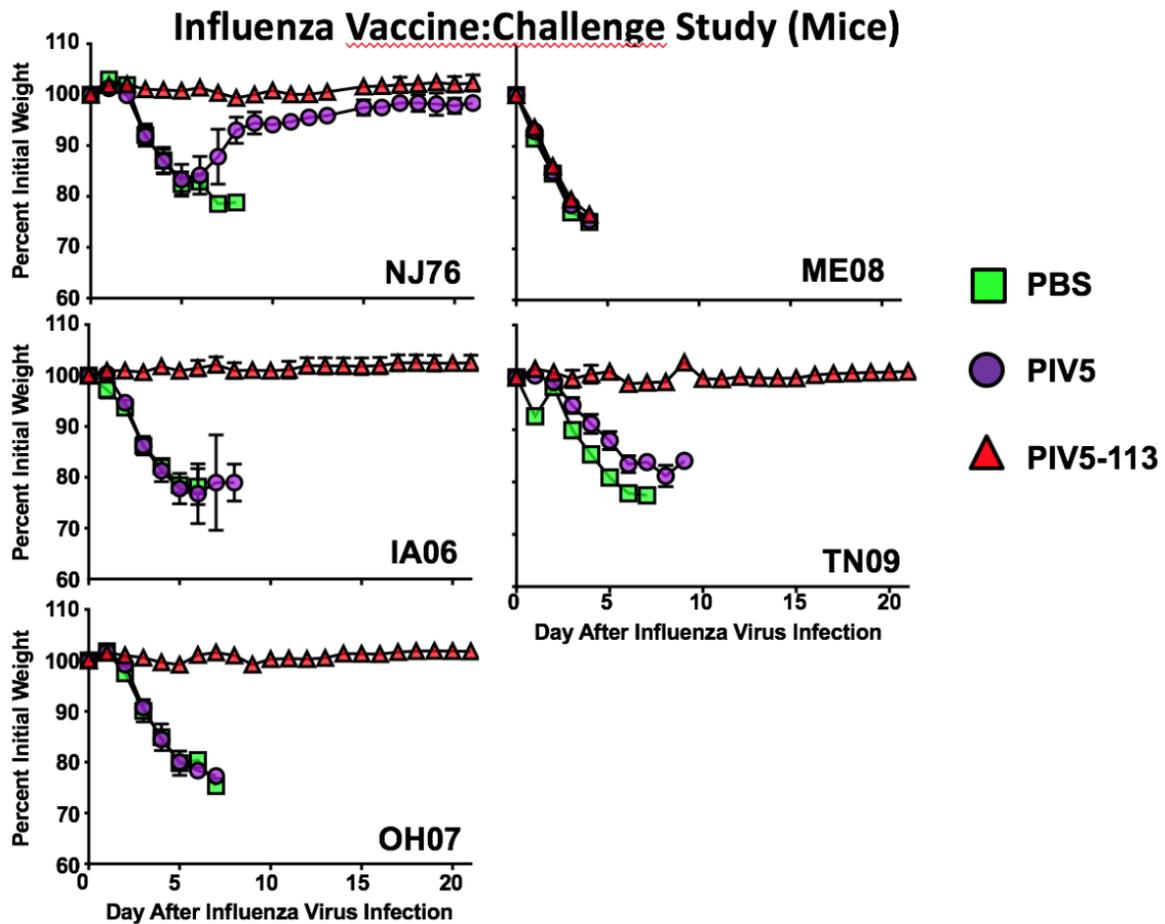
Figure 4: This graph shows the influenza vaccine:challenge study in mice. Protective immunity established. The numbers of mice per challenge were as follows: 4 per challenge for PBS, 8 per challenge for PIV5, and 7-8 per challenge for PIV5-113. Data provided by Sarah Zaiser.
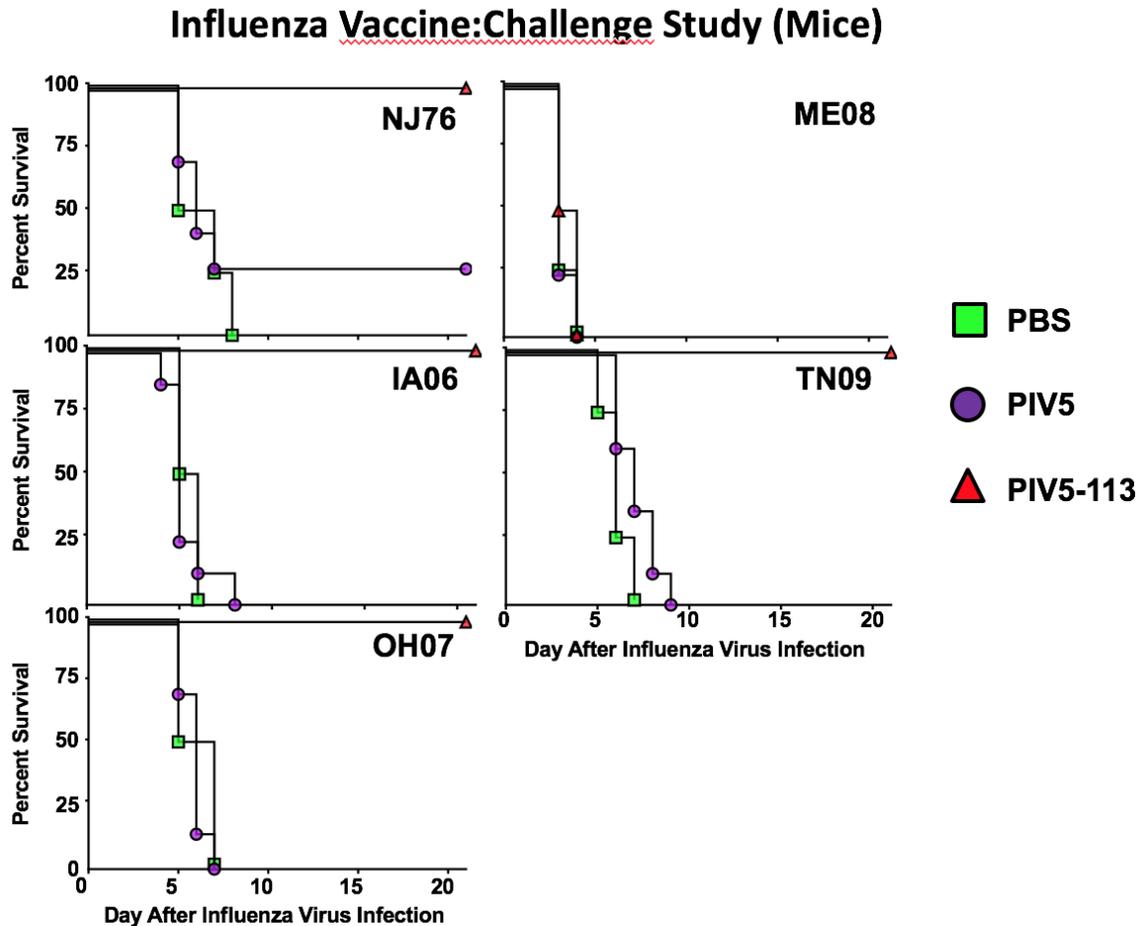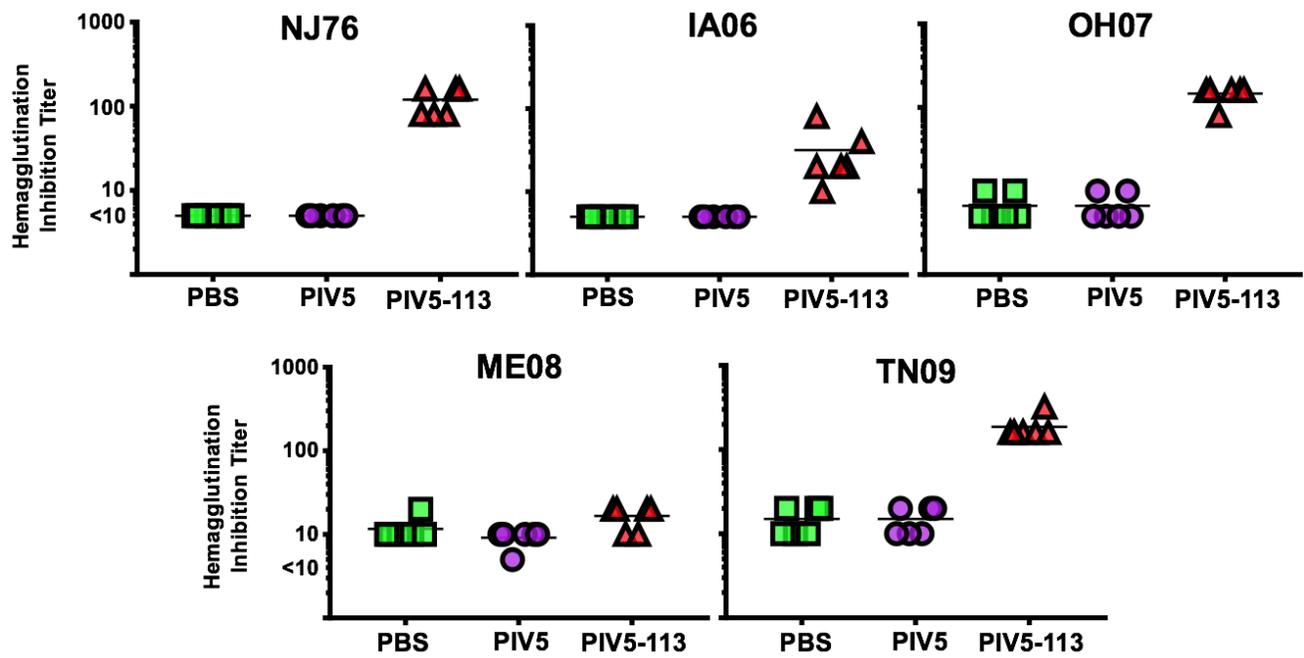
Figure 5: This graph shows the influenza vaccine:challenge study in mice. Protective

immunity established. The numbers of mice per challenge were as follows: 4 per

challenge for PBS, 8 per challenge for PIV5, and 7-8 per challenge for PIV5-113. Data

provided by Sarah Zaiser.

**Hemagglutination Inhibition After Vaccination
Pig Study (Day 5 Post-Challenge):**

Figure 6: HAI assay results after vaccination in pigs. There were 6 pigs per vaccine group. ME08 showed no antibody detection, but NJ79, IA06, OH07 and TN07 all had antibody detection after vaccine delivery.

Hemagglutination Inhibition After Vaccination
Pig Study (Day 5 Post-Challenge)
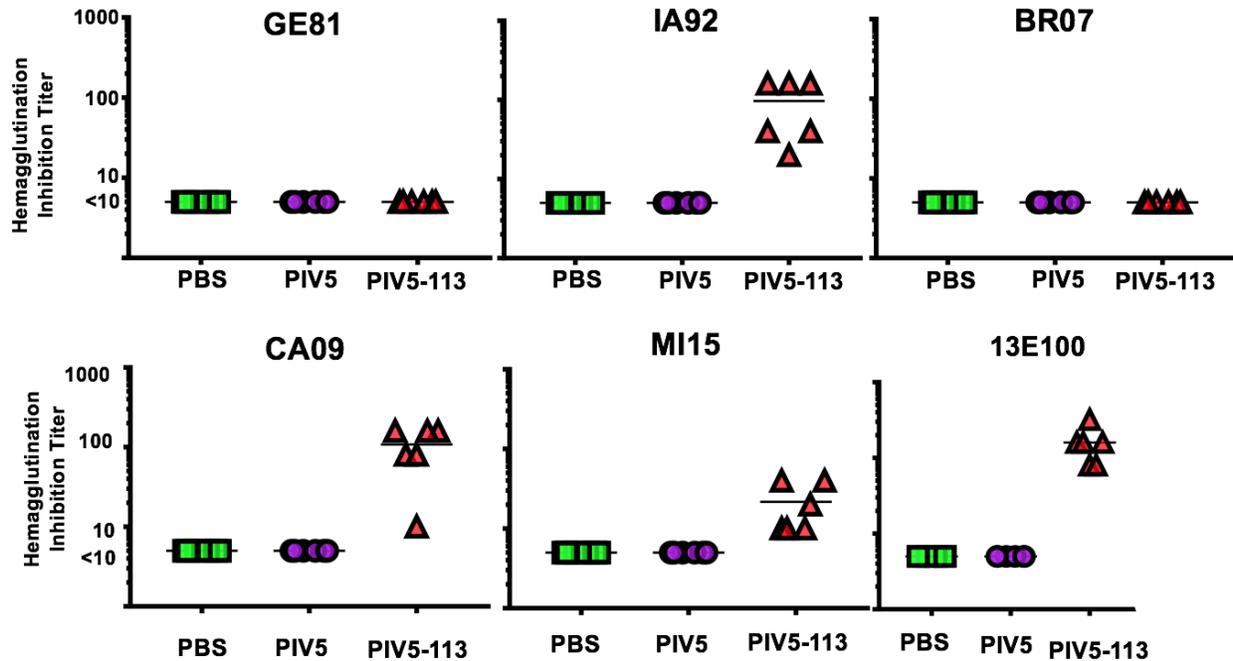
Figure 7: HAI assay results after vaccination in pigs. There were 6 pig per vaccine group. IA92, CA09 and MI15 all had antibody detection after vaccine delivery, but BR07 and GE81 did not.

Chapter Five

Discussion and Summary

Currently influenza prevention depends on predicting which variants of the virus will circulate throughout the year and then creating vaccines that protect against those variants. The World Health Organization is responsible for selecting three strains that will resemble the ones circulating during influenza season in the Northern and Southern hemispheres (CDC, 2018). Pandemics (antigenic shift) and epidemics (antigenic drift) can be misinterpreted, and we have been poorly prepared for these situations when they arise. Our PIV5-113 vaccine provides sufficient data to give hope to the possibility of a universal influenza vaccine. This single vaccine induced immunity against influenza viruses that circulated in pigs and humans both before and after the novel H1N1 virus that caused a pandemic in 2009. With its creation, and successive improvements in both design and immunogenicity, pandemic and epidemic influenza infections can be prevented.

Pandemics represent a more rapid and dramatic change in the genetic makeup of viruses through re-assortment of the individual virus genes, and they usually come from a known source, such as another species like pigs or birds. When influenza viruses cross from animals to humans, novel or "pandemic" stains are be generated (Berlanda Scorza, F., et al., 2016). According to the CDC, "An influenza pandemic is a global outbreak of a new influenza A virus. Pandemics happen when new (novel) influenza A viruses emerge which are able to infect people easily and spread from person to person in an efficient and sustained way" (Pandemic Influenza, n.d.). Alternatively, epidemics arise through an accumulation of point mutations that arise as

the virus evolves to escape adaptive immunity and they lead to annual vaccine changes.

In cases like the 2009 H1N1 influenza pandemic, a vaccine might need to be created after the strains for the season have already been selected (Huber VC, et al., 2009). Based on the statistics for deaths and hospitalization provided by the CDC, the method currently used is clearly not very efficient, especially during the initial wave of a pandemic. Viruses in birds and pigs can be studied before they emerge in humans. If this knowledge is available, a pre pandemic vaccine can be created. Using PIV5 and creating an HA like HA-113, which was created using DNA shuffling, broad immune responses can be induced. Four phylogenetic clades were represented by the chosen parental influenza A H1N1 strains, covering those known to circulate in pigs. HA genes of these parental strains (NJ76, IA06, OH07, ME08, and TN09) were used for DNA shuffling and here we evaluated the HA-113 chimeric construct created. PIV5 was utilized as a vector for expression of this candidate chimeric HA protein. The data presented here show that HA-113 expressed in PIV5 can induce immunity against HA proteins representing parental HAs that are included within the DNA-shuffled sequence and that the PIV5-113 vaccine induced antibody titers at levels that protected against NJ76, IA06, OH07, and TN09 virus infections. Antibodies were also induced against ME08 when the PIV5-113 vaccine was used, but these did not protect against challenge with an ME08-expressing virus. It is worth noting that this virus circulated in humans prior to 2009 and was much different than the other viruses used in DNA shuffling.

It may be possible to create a universal vaccine using DNA shuffling, which creates recombined chimeric HA proteins. By incorporating HA epitopes from swine

and human influenza viruses into the vaccine, this could be utilized as a pre pandemic vaccine to decrease the amount of people infected with this virus. The HA epitopes should allow the vaccine-induced antibodies to recognize the circulating virus and allow for the recognition of viruses that evolve later. The prime example of this is how PIV5-113 induced antibodies against a virus isolated from pigs in 2013 (13E100) and the human virus from 2015 (MI15). This indicated that the immunity against the evolved HAs is maintained by the chimeric HA vaccine. By creating a vaccine that can prevent the virus from circulating around the swine species, this could lessen the chances of a pandemic like the one that occurred in 2009.

According to the CDC, "Improved surveillance of influenza in pigs and other animals may help to detect the emergence of influenza viruses with the potential to cause illness and spread among people, possibly resulting in a pandemic. Early detection of such viruses can alert public health officials and aid in pandemic preparedness through the development of appropriate diagnostic tests and influenza vaccine candidate viruses, if necessary" (Origin of 2009 H1N1). By using DNA shuffling, we were able to create a vaccine that induced enough antibodies to provoke an immune response.

Creating chimeric HA's of the H1N1 subtype via DNA shuffling is a much more proactive solution than the current method. Since the HA construct showed immunogenicity in both mice and pigs, it is hypothesized that it could induce protective immunity amongst the circulating variants in humans. The MI15 virus, which is a virus from 2015 that was isolated after the 2009 epidemic, was recognized by antibodies induced by PIV5-113. This indicates that other viruses from after 2009 should be tested

to see where and when the degree of PIV5-113 reactivity ends. If a vaccine is developed using this approach, pandemics will hopefully be avoided, and vaccines could target both pigs and humans to limit interspecies transmissions that are associated with pandemics. In the DNA-shuffling approach a new chimeric HA can be created and tested when the PIV5-113 no longer induces sufficient antibodies.

In summary, the current method of developing vaccines is too slow to prevent pandemics (Robertson JS, et al., 2011), but the PIV5-113 vaccine tested here showed antibody responses in mice pigs that predict similar performance in humans. This means that a vaccine like PIV5-113 may limit transmission events between pigs and humans, which would be important either before a pandemic or early during the pandemic. If this would have been available in 2009, the pandemic could have been lessened or avoided completely. Hopefully my work in Dr. Huber's laboratory contributes to the possibility of developing a universal vaccine with broad immunity.

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