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Review of a Chimeric Hemagglutinin-Based Influenza Vaccine Approach

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Abstract

Influenza virus has affected human populations for centuries, causing an acute respiratory effect on the body. Even with scientific advancements in vaccine research, there are still serious limitations to the influenza vaccine. Varying strains and the constant recombination of the influenza virus has caused annual influenza circulation to be difficult to predict. The ongoing race to develop a more efficient vaccine to broadly protect against the variety of influenza strains remains a public health priority. Many strategies have been studied to improve the current vaccine. The use of a chimeric hemagglutinin-based vaccine is in the first stages of human trials and is proving to be a promising method in the development towards a universal influenza vaccine.

One Sentence Summary: A systematic review of the research surrounding a chimeric hemagglutinin-based approach for influenza vaccine development.

Abbreviations: CDC=Center of Disease Control; cHA=Chimeric Hemagglutinin; IV= Influenza Virus; IAV=Influenza A Virus; HA=Hemagglutinin; NA=Neuraminidase; NP=Nucleoprotein; RBS=Receptor Binding Site; WHO=World Health Organization

Introduction

Influenza virus (IV) circulates across the globe causing an annual disease burden to millions of individuals. Every year different strains of IV spread throughout the population causing illness, hospitalization, and death. IVs are particularly severe in high-risk groups including the immunocompromised, elderly, pregnant women, and health workers. Currently, the most effective way to control the severity of the seasonal influenza season is with vaccination. (1,2). Influenza vaccines must be updated by researchers yearly based on the present circulating strains. The vaccines selected each season are a prediction based on previous data collected on influenza. Occasionally the effectiveness of the influenza vaccine drops due to poor predictions or the appearance of a novel strain. Improving the effectiveness of the vaccine to drop annual mortality due to the virus is a pressing public health concern. Continuous research on developing a broad protecting universal influenza vaccine has led to a variety of methods being considered for human trials (1,3,4).

The issues facing current influenza vaccinations is the narrow protection against the virus. Even the most recent vaccines mostly target the dominant head domain of the virus's hemagglutinin making the vaccine strain specific. The hemagglutinin head group can allow the virus to escape neutralization because it is highly plastic allowing for an increase rate of mutation. This is what gives rise to influenza pandemics with a novel strain of pathogen. There are many possible methods researchers are studying to create a vaccine that avoids the virus's specific hemagglutinin head domain. The development of a vaccine that is not strain specific can give individuals protection against all circulating influenza strains, increase the overall seasonal vaccination effectiveness, and decrease the annual mortality rate due to the influenza virus (5,6). A promising method currently in the first phases of human trial is the chimeric HA-based

vaccine approach, which can target a conserved region of the IV hemagglutinin and avoid the high plastic globular head domain of the virus (6,7).

Current Influenza Vaccines

Vaccinations are the most effective way to protect against the influenza virus and are recommended by public health organizations as the main form of protection against influenza associated complications. The influenza vaccine can be less than 60 percent effective depending on the annual influenza season. With major faults in our current vaccine strategy, it is still our best defense against the yearly outbreaks. Ongoing research to improve the efficiency of the vaccine can significantly impacted the spread of the disease (8).

Currently, the influenza vaccine is administered annually and is necessary to keep up with the newly emerging strains of influenza. The antibody response induced by traditional vaccines is fleeting. A systematic review of various influenza vaccinations showed the effectiveness of vaccines waned 180 days after vaccination. This means the immune response fades only 6 months after receiving an influenza vaccination. The variation of the virus and the fading immune response cause narrow protection against the virus (8,9).

Another problem associated with current vaccination strategies is the lack of protection against newly emerging influenza strains like the 2009 H1N1 outbreak. Development of influenza vaccines take around 8-9 months this includes testing and producing enough vaccines to distribute worldwide. When a new strain of virus emerges, the vaccines are released almost a year later and by that time it is too late to offer protection (10,11). The vaccines can cover around 3-4 strains at a time, but the continuously shifting virus requires a broader more efficient vaccine to offer effective protection (12).

Influenza Virus

In order to create a broad protecting vaccine, researchers must understand the structure of influenza. Influenza infection in humans is primarily caused by influenza A virus (IAV), but infection can also be caused by influenza B virus (IBV). Both, IAV and IBV, are enveloped RNA viruses (1). IAV has several different strains making it the more difficult virus to vaccinate. Two important proteins on the surface of the virus include hemagglutinin (HA) and Neuraminidase (NA), which are key to host cell entry and infection (Figure 1). Figure 1 also shows the RNA-binding matrix protein M1, nucleoprotein (NP) coating the viral RNA and the ion channel M2 protein. These components can be detected by the immune system, but most neutralizing antibodies target the globular head of HA. The HA and NA are prominent on the surface of the virus and are accessible targets than the other components (Figure 1) (1,7,13,14,15).

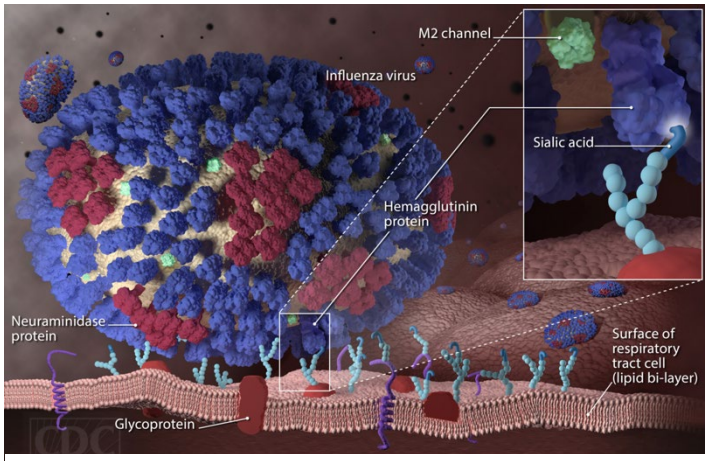

Figure 1: Structure of influenza virus and its interaction with host cell membrane (13).

The HAs found on the surface of the virus are made of the globular head domain and the stalk domain. The HA head domain is the major surface glycoprotein of the IV. The immune response is mostly induced against the head domain, which is highly variable causing the current vaccinations to be narrow in protection against IVs (7,16). An individual develops specific neutralizing antibodies for the strain's HA globular head domain, when exposed to IAV. The HA head is subject to random mutations causing the strains to vary over time. The antigenic drift of

these HA protein sequences makes pre-existing antibodies useless against the new variants (7,15).

Another challenge associated with IV variability is antigenic shift and recombination. Recombination occurs when two strains of IV infect a host and yield a new HA completely foreign to the immune system. Antigenic shifts have damaging effects to the human population because the new strains have been known to cause global pandemics (1,7). For example, the H1N1 outbreak in 2009 caused an estimated 150,000-575,000 deaths and approximately 60.8 million infections (7,14). Organizations like the Centers for Disease Control and Prevention (CDC) are critical for collecting and analyzing IV data so researchers can predict the strains needed for vaccinations. The design of current vaccinations is the reason for staggering vaccine efficiency from year to year (7). Antigenic variation is found in most viruses, but the degree of variation ranges extensively. IV experiences more antigenic variation than other viruses like measles and polio. Unlike IVs, measles and polio offer lifelong immunity once exposed because the variation is small. Although, once exposed to a strain of influenza the immunity is long-term for the specific strain. The rapid and constant antigenic variation diminishes the success of a long-term immunity (15).

To combat the frequent antigenic drift in the globular HA head domain of the IV, researchers are looking to target epitopes that are less mutationally tolerant. The HA stalk region of IVs is a current target of vaccine development research (15). The purpose of the HA is to mediate the binding of the virus to the host cell via the receptor binding site, while the stalk domain assists in the fusion between the virus and endosomal membrane. The virus must retain the structure of the binding site and stalk domain in order to stay viable. These two regions of the IV HA are relatively conserved and are similar across all IAV strains (11).

The stalk domain being more conserved than the globular head domain, it evolves at a much slower rate. The importance of the HA stalk domain for vaccination development lies in the neutralizing B-cell epitopes. The stalk epitopes are conserved among influenza A virus subtypes. The conserved regions correspond to preserved enzymatic activity (polymerase or protease), or structural features that will not be easily changed without deleterious effects. Research from Erika Kirkpatrick et al. evolutionary analyzed stalk domains of the HA from H1N1, H3N2, and influenza B viruses. The analysis showed the stalk domain evolves at a significantly slower rate than the head domain. Additionally, the research found the cross-reactive epitopes in the stalk domain, that are a target for broadly neutralizing monoclonal antibodies, evolve at an even slower rate compared to the full HA protein (head and stalk domain). (17). The use of the HA stalk region in vaccine development has researchers analyzing many promising approaches. The chimeric HA-based approach is one of the current strategies being utilized in the race towards a long-lasting and broad protecting influenza vaccine (3, 17).

Chimeric Hemagglutinin-Based Approach

The chimeric hemagglutinin (cHA)-based approach is one of the most common methods explored in the development towards a universal influenza vaccine. This approach takes advantage of antibodies that cross-react against different groups of influenza. More specifically anti-stalk antibodies have been shown to create an immune response against the influenza virus. These antibodies act by neutralizing hemagglutinin activation and neuraminidase activity of the virus through steric hinderance (3,18). cHA-based approach is a solution to bypass the epitopes on the variable head region on the HA and target more conserved epitopes on other regions of the virus. If researchers can create an immune response against the regions undergoing little

mutation, the annual influenza vaccine can offer near-universal protection against influenza (6, 11).

Immunodominance of HA increases the difficulty of creating an antibody response strong enough for the stalk domain (19). Stalk antibodies are rare or not found in individuals vaccinated with current IV vaccines. However, stalk antibodies can be detected after being infected by H1N1 and H3N2 IV strains naturally (In both human and mice models). (11). Many proof-of-principle studies found stalk antibodies can neutralize IAV and protect against infection. HA-stem antibodies are one of the more promising approaches for improving the annual IV vaccine (20).

Chimeric HA proteins have the same stalk of the widely circulating IAV strain (H1 clade) and it is fused with the globular head of a non-human IAV strain. For example, an H5 head and an H1 stalk HA can be fused to form cH5/1. This construct allows for proper folding and stabilization of conserved HA epitopes and is a useful tool for detecting the stalk antibodies in clinical research (21) The goal of the cHAs is to generate stalk-specific antibodies to provide the broad protection against all IAVs. For the cHA to target accessible regions during an infection, researchers use epitope mapping of the stalk immunogenic sites to design a proper vaccine. Using the cHA approach allows the immune system to create antibodies against non-human HA head domains and the stalk-specific antibodies will provide the immune protection (7,21).

The target epitope for the stalk-specific antibodies can be found in the proximal-membrane stalk region and they do not inhibit the virus from binding to the host cell. The stalk antibodies are still able to neutralize virus activity. Researchers found through structural modelling; the antibodies bind to a hydrophobic patch in the helical bundle of the stalk region. The helical bundle in found on the proteins tertiary structure. An experiment indicated

conformation-sensitive recognition of stalk antibodies and the HA, because the binding was inhibited by low pH and reducing agents. These findings suggest the stalk antibody binding to the influenza HA induce a conformational change that prevents virus fusion with the host cell (22) Understanding the mechanisms of the stalk antibodies is beneficial for determining which antibody subclasses will provide the most useful vaccine (21,22).

Chimeric Hemagglutinin-Based Approach Animal Models

Before researchers can consider applying this vaccine to humans, they first use animal models to test the concept of the vaccine. A variety of models were used before researchers started the first phases of human clinical trials. The different models include mouse, ferret, primates, and guinea pig models. The animal model experiments are used as proof-of-concept in the development process because humans have a significantly different immunological history than the animals used in these studies. Vaccination strategies like the use of cHA are influenced by previous immunity. Humans are exposed to influenza many times throughout their lifespan, and many have taken a variety of influenza vaccinations. How everyone develops an immune response against the IV can vary greatly (11). The models still provide key insight in vaccine design and function (23). The goal of testing cHA-based vaccines with animal models is to create a sequential vaccination strategy that limits the immune response to the head HA region and focuses on the stalk epitope targets. The vaccine needs to be broad, long-lasting and should be able to be applied to humans (11,23).

In a recent study by Hsin-Yu Liao et al., mice were infected with influenza H1N1 and titers of broadly neutralizing antibodies that target the hemagglutinin stem region where measured. Chimeric Hemagglutinin with a DNA prime protein boost to the stem region

vaccination produced broadly protective stem antibodies in the mice model. Along with chimeric hemagglutinin, soluble trimeric hemagglutinin vaccine with stem subunit was shown complete protection in mice against the virus. The development of DNA vaccines and their use of stem-specific antibodies is still current, they are extremely promising in the search for a new influenza vaccine (24).

Similar studies used the cHA and tested different existing vaccination strategies including adjuvanted, live-attenuated, and inactive vaccines. In one study using mice, adjuvanted and attenuated with cHA constructs were found to induce a broad immune response compared to other vaccine strategies. This concluded adjuvanted and attenuated would-be useful strategies in human models to induce high enough stalk antibodies for virus neuralization (23). Other researchers suggest using all three vaccine strategies in a human clinical trial to find the most efficient method (11).

Other research looks at the neutralizing activities of the cHA vaccines in mice found the Fc region of the antibodies is an important mechanism for the neutralization of the virus. Fc-FcR between the stalk antibodies and the HA are important for an immune response. Multiple experiments using the cHA vaccine strategy show stalk specific antibias contribute to direct IV neuralization or Fc-mediated neuralization. This has a significant impact in how researchers continue to design the vaccine because the mechanisms of stalk antibodies play an important role in the immune response (23,25).

Overall, animals provide a great model in the processes of designing an effective cHA vaccine that can have broad and long-lasting effects on circulating influenza. Animals are important tools in the race towards a universal vaccine because they provide key insight into the underlying mechanisms responsible for infection and neuralization of the virus. The goal is to

take the research obtained from the animal models and be able to apply it in human clinical trials. For vaccines to fight against the annual influenza strains and induce disease preparedness they need to be fully understood and tested in human participants (26).

Chimeric Hemagglutinin-Based Approach Clinical Trials

The use of animal models is beneficial for understanding the overall concept of the vaccine, but its effectiveness in humans will vary drastically from the animal models. Given humans have a different immunological history of influenza than the animal models and everyone has been exposed to varying strains and vaccinations throughout their lifetime. The mechanisms in which anti-stalk antibodies interact with the influenza virus have been explored in human clinical trials by Kaori Sano et al., showing the antibodies neutralize the virus in two modes. The antibodies either bind via fab paratope binding to the hemagglutinin stalk region or they bind via Fc glycan binding to the hemagglutinin receptor binding site (RBS). The study found the mechanism responsible for virus neuralization is IgA polymerization (27). The findings of the previous study were supported by another clinical trial viewing broadly neutralizing HA stalk antibodies. The antibodies rely on engagement of the Fc-Fc receptor in the epitope region. This type of interaction is only found in the stalk neutralizing epitopes and not in the globular head region of HA (28). The importance of these findings aids researchers in creating the correct design for cHA-based influenza vaccines.

The phase I clinical trial of the cHA-bases approach proved promising in the recent development of this novel vaccine. This study performed by Bernstein et al. was a randomized observer bind trial in healthy adults. Their where two placebo groups and one cHA-based vaccine. 65 participants were randomly assigned, and anti-hemagglutinin stalk titers were

measured. The experiment showed the chimeric hemagglutinin-based vaccine produced a cross reactive serum of IgG antibodies that specifically targeted the stalk domain. This trial proved this can be a vaccination for human use with high enough anti-stalk titers to neutralize the influenza virus (29).

A similar study used the same concept with a pandemic IV vaccine by using cHA to boost the amount of stalk antibodies. Adjuvanted, inactivated, and live-attenuated vaccines were used in the administration of the stalk antibodies. The study used a cH6/1, and the antibodies titers were measured via ELSIA (figure). Full length H2 and H18 HA antigens were used to measure the antibodies in the ELISA. The vaccines contain IAV H1N1, H5N2 and H9N2 influenza strains and tested the effectiveness in healthy adults. The cHA vaccine was able to induce high titers of H1 stalk antibodies. When participants received an adjuvanted and inactivated cHA vaccine, there was a significantly high level of stalk antibodies, even after one dose. Another important finding in this study was the longevity of the stalk antibodies measured in the ELISA. The titers initially decreased after the first vaccination but were able to stabilize over baseline. The antibodies were well over baseline 6 months after administration and remained consistent after 18 months. A longer study needs to be explored to examine the exact longevity of the antibodies, and how the antibodies defend against the virus compared to the current influenza vaccination (30).

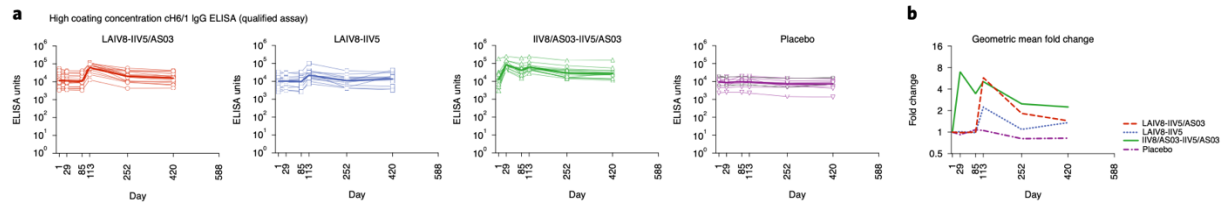

Figure 2: Serum stalk-specific antibody IgG titers in the LAIV8-IIV5/AS03, LAIV8-IIV5, IIV8/AS03-IIV5/AS03 and placebo groups as measured against recombinant cH6/1 HA substrate in a qualified assay (30).

Many vaccine production companies are working towards the advancement of cHA-based vaccines. GlaxoSmithKline for example is working on clinical trials of two different cHA vaccines. One of the cHA vaccines is being collaborated between GlaxoSmithKline and the Icahn School of Medicine and Duke University. Another company known as Novavax, is on its 3rd phase of human clinical trials with a cHA vaccine called Nanoflu. The goal of the continuing trials is to test the efficiency of the vaccine on people over the age of 65, which is a critical group for influenza complications (7,30). The first phases of these clinical trials support cHA vaccines proof-of-concept and encourage researchers to further develop this strategy (30,31,32)

Safety of Chimeric Hemagglutinin-Based Approach

HA stalk-binding antibodies have been tested in several human trials to prove their safety and effectiveness in vaccinations against influenza. In a recent phase I human trial of the cHA-based vaccine, no safety concerns were reported by the 65 participants ages 18-65 (Bernstein, 2019). The problem with the human trials of the hemagglutinin stalk approach is they are performed on individuals 18-65 years old with no major health concerns. Further testing needs to be done to understand the level of protection the vaccine gives the at-risk population like immunocompromised and 65 years and older individuals (33).

Another phase I human trial was used to test the safety of using a variety of influenza vaccines with a cHA base. The trial found that the dosage required to induce a broad long-lasting stalk antibody provided safe use in healthy adults 18-65. The only reactions reported in these individuals were pain and redness in the injection site and fatigue up to 4-7 days after administration. This human trial focused on IAV strains so further research on the safety of IBV strains is still needed to prove total safety. The experiment found combining cHA into a trivalent

or quadrivalent vaccine for the influenza season would be safe for healthy adults. Both human trials looking at the safety of this vaccine strategy supports the further development of this vaccine. (31,32,34)

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Implications

The most challenging problem with the influenza virus is the continuous antigenic drift and shift of the virus strains. The divergence of strains causes catastrophic pandemics like the outbreak of H1N1 that infected millions of humans across the globe. Current influenza
320 vaccinations must be carefully planned each season to predict the circulating strains present all over the world. Even with researched predications there is still no protection against newly emerging strains. For example, the H1N1 pandemic of 2009 cause thousands of deaths and millions of infections (34,35). Creating a universal vaccine with broad protection across all strains eases the fear of antigenic drift and shift of the virus strains. Annual protection against all
325 strains of influenza also relieves researchers from having to prepare and predict the annual influenza vaccine 8 months in advance each season (35).

Specifically, using antibodies targeting the conserved stalk domain of influenza in the novel hemagglutinin-based approach brings researched one step closer to a universal influenza vaccine. Antibodies targeting the hemagglutinin head group, like the current annual vaccinations.
330 have limited capabilities because mutations are frequent in the virus head group. These stalk antibodies are of recent interest to researchers as the key to an improved annual vaccination. (36). The cHA approach has the potential to protect against all varying IAV strains and can prevent pandemic outbreaks from infecting millions of individuals. The non-human HA head used in the cHA will allow the immune system to develop strain-specific antibodies to the

conserved stalk region. A potentially lethal infection for the immunocompromised can be avoided (34,36).

Limitations

Current limitations slow the development of a universal influenza vaccination. While using stem specific antibodies in influenza vaccine development has brought research closer towards a broader immunization. Humans do not produce a high concentration of stem specific antibodies in a natural immune response. Research by Hyon-Xhi Tan et al. found B cell responses that are stem-specific are exceedingly subdominant during the initial infection (37).

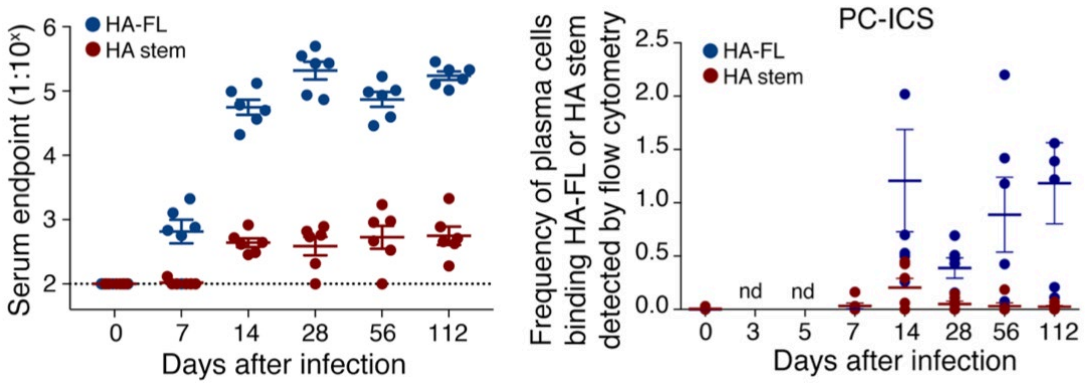

Figure 3: Serum endpoint total IgG titers measured by ELISA in mice infected with H1N1 influenza. Frequency of plasma cells binding full-length hemagglutinin (HA-FL), or stem hemagglutinin (HA stem) antibodies detected by flow cytometry by days after infection (37).

Figures from Hyon-Xhi Tan et al. research convey the limitations of stem antibodies attack on influenza during infection (figure 2). The full-length hemagglutinin attacking the strain specific head group of influenza experiences a much higher frequency of B cells and plasma cell response. The level of protection with HA-FL compared to HA stem is higher with current vaccinations. With HA-FL creating a higher level of protection against influenza it makes producing vaccines with them a safer choice. The neutralizing antibodies of influenza have been

found to predominantly effect the hemagglutinin globular head group. Over the recent years, research on other neutralizing antibodies like the stem hemagglutinin have been greatly improved, but there is still work that needs to be done to replace the current vaccine. (35,37).

Even though many experiments show proof-of-concept for the cHA approach more clinical human trials need to be performed to fully understand the efficiency of the vaccine. Human models have certain intrinsic limitations. The dosage effects of cHA vaccines vary significantly from animal to human models. The animal models never fully capture the immunological history of human subjects. Current human clinical trials of cHA vaccines found safety in the approach but longer studies to find the long-term efficiency of the vaccine still needs to be researched (3,7,30). It is clear substantial progress has been made in the development in a more efficient influenza vaccine, but there is still more research needed to do to achieve a long-term solution for influenza (38,39,41).

Conclusion

To summarize, influenza is constantly evolving, and the highly plastic globular head region makes it hard for researchers to predict and create an effective annual vaccination. The current influenza vaccine only provides strain-specific immunity that only provides short-term protection against the fast evolution of the virus. To reduce the continuous virus monitoring, vaccination adjustments and strain predictions, researchers are on the search for a universal influenza vaccine. The cHA-based approach brings researchers one step closer to developing a more efficient and near universal vaccination. Even though, current influenza vaccinations are the main prevention strategy against the virus, influenza is still able to cause seasonal epidemics

globally. The current research towards a broad protecting and long-lasting vaccine can significantly reduce influenza's worldwide burden on the human population.

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