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T cell epitope engineering: an avian H7N9 influenza vaccine strategy for pandemic preparedness and response

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ABSTRACT

The delayed availability of vaccine during the 2009 H1N1 influenza pandemic created a sense of urgency to better prepare for the next influenza pandemic. Advancements in manufacturing technology, speed and capacity have been achieved but vaccine effectiveness remains a significant challenge. Here, we describe a novel vaccine design strategy called immune engineering in the context of H7N9 influenza vaccine development. The approach combines immunoinformatic and structure modeling methods to promote protective antibody responses against H7N9 hemagglutinin (HA) by engineering whole antigens to carry seasonal influenza HA memory CD4+ T cell epitopes – without perturbing native antigen structure – by galvanizing HA-specific memory helper T cells that support sustained antibody development against the native target HA. The premise for this vaccine concept rests on (i) the significance of CD4+ T cell memory to influenza immunity, (ii) the essential role CD4+ T cells play in development of neutralizing antibodies, (iii) linked specificity of HA-derived CD4+ T cell epitopes to antibody responses, (iv) the structural plasticity of HA and (v) an illustration of improved antibody response to a prototype engineered recombinant H7-HA vaccine. Immune engineering can be applied to development of vaccines against pandemic concerns, including avian influenza, as well as other difficult targets.

Introduction

The need to prepare for infectious disease outbreaks to prevent catastrophic loss of life and societal disruption drives innovative vaccine development. New technologies present opportunities to meet the challenge faced when long standing technologies do not yield effective vaccines. Here, in the context of avian H7N9 influenza, we present a novel antigen design strategy combining immunoinformatic and structure modeling technologies to harness both T cell and B cell immune mechanisms to produce more effective vaccines.

Concern for an avian H7N9 influenza pandemic

In 2013, the first cases of human infection with avian influenza A (H7N9) were reported in mainland China.[1,2] Since then, China has experienced five epidemics of human infection with H7N9.[3,4] Outbreaks typically occur in a seasonal pattern peaking during January-March and dropping off by end of May. This trend, however, may be changing. The fifth outbreak of H7N9 began in October 2016 with a spike in cases in December.[4] Along with an earlier onset of reported cases, there was also a sudden increase in human H7N9 cases reported.[3,5,6] Of the total number of human H7N9 infections identified since 2013, 52% of cases have occurred during the latest outbreak.[7] As of March 2018, a total of 1,567 cases of

laboratory confirmed H7N9 infections were detected with at least 615 deaths reported.[8] The high case fatality rate associated with avian H7N9 infection poses a continuing threat to human health. In addition to an increase in H7N9 human infection, the cases reported have spread into western provinces for the first time.[3–5] Changes in distribution were also noted during the fifth wave from affecting mostly elderly to middle aged adults, as well as an increase in cases from urban areas to suburban and rural areas.[3,5]

Human H7N9 infections have been largely zoonotic through exposure to infected poultry.[9] An epidemiological study in China between May 2013 and May 2014 showed 6.7% of case contacts developed H7N9 antibodies, suggesting that human-to-human transmission occurs and could cause mild or asymptomatic infection.[10] Both human-to-human household and hospital clusters have been described.[11,12] Transmissibility of avian influenza to humans depends on a balance of activities of the viral surface glycoproteins HA and neuraminidase (NA). The vast majority of human H7N9 isolates bears the hallmark Q226L mutation in HA that confers human receptor binding (α-2,6-linked galactose) and reduces avian receptor binding (α-2,3-linked galactose).[13] N9-NA also demonstrates receptor binding properties with a preference to binding human α-2,6 linkages.[14] Of note, before the fifth outbreak, human infections were caused by a low pathogenic avian influenza, which caused little or no disease in infected

poultry.[6] However, in February of 2017 the National Health and Family Planning Commission of China reported genetic sequences from virus isolates from two patients located in Guangdong Province that had insertions at the HA gene cleavage site which is suggestive of a highly pathogenic avian influenza.[6,7] Additionally, mutations in the viral polymerase that may augment replication and pathogenicity in humans have been observed, however human cases of highly pathogenic H7N9 virus infection do not exhibit increased transmission and virulence.[15] Combined with already existing adaptations in H7N9 for increased viral replication in the mammalian lower respiratory tract[16], the potential for further adaptations that increase human-to-human transmissibility raises concern for an H7N9 influenza virus pandemic, despite lower incidence in recent months than in previous years.

Challenges to preparedness for an avian H7N9 influenza pandemic

Historically, vaccination has been the most effective strategy to control seasonal influenza spread and is the basis for efforts to develop avian influenza vaccines. Relative to seasonal influenza, vaccination against avian influenza poses a unique challenge because the human population is immunologically naïve.[17] Vaccination cannot rely on preferential recruitment of memory B and T cells to elicit a protective antibody response due to distant sequence relatedness with seasonal influenza. Any cross-reactive memory B and T cells would be present at frequencies too low to confer protective antibody immunity by seasonal vaccination.[18] As a consequence, avian influenza vaccines require higher doses than seasonal vaccines or adjuvant formulation to stimulate robust immune responses.[18]

H7N9 influenza HA elicits weak neutralizing antibody responses in natural infection and vaccination. H7N9-infected humans display delayed development of the correlate of influenza protection, hemagglutination inhibition (HI) antibodies, when compared to seasonal influenza infection; IgG avidity for H7N9 HA is also significantly lower.[19] One report showed only 10% of subjects seroconverted in a Phase I trial of a live attenuated H7N9 vaccine.[20] The results of alternative vaccine strategies have also been subpar with both inactivated H7N9 split vaccine and H7N9 HA virus-like particle vaccine eliciting HI seroconversion rates of only 6% and 15.6% in Phase I trials respectively.[21,22] This compares poorly with the ~ 89% rate reported for similar seasonal influenza subunit vaccines.[23–25] Even with the addition of adjuvant and a booster injection, the inactivated split H7N9 vaccine elicited HI seroconversion in only 59% of subjects in an early Phase II clinical trial.[26] More recently, a two-dose AS03-adjuvanted inactivated virus vaccine showed 86–96% seroconversion for different antigen doses and adjuvant formulations three weeks after the boost immunization but these rates dropped off significantly by the six-month time point.[27] Compared to seasonal H1-HA and H3-HA, fewer CD4+ T cell epitopes have been found in H7N9 HA.[28] Additionally, conservation of T cell epitopes with other strains of influenza was very limited[29]. To better prepare for an H7N9 influenza pandemic, vaccine strategies that overcome the poor immunogenicity of novel H7N9 HA are needed. To address this challenge, T cell epitope engineering of HA aimed at recalling memory CD4+ T cell to seasonal influenza is a promising strategy that may be utilized to create a vaccine with improved immunogenicity and efficacy.

Memory CD4+ T cells contribute to protection against influenza

The role memory CD4+ T cells play in pandemic influenza A H1N1 (pH1N1) immunity demonstrates the potential to harness pre-existing seasonal HA-specific CD4+ T cells to produce a more potent H7N9 vaccine. A study of human pH1N1 infection in individuals with no pre-existing protective antibodies showed that cross-reactive memory CD4+ T cells correlate with lower virus shedding and less severe illness.[29] Similarly, mice exposed to pre-pandemic H1N1 develop memory CD4+ T cells that protect against pH1N1 infection.[30] In clinical vaccine studies, a single dose of monovalent pH1N1 vaccine generates neutralizing antibodies, suggesting that memory CD4+ T cells are able to support naïve B cell responses to a novel HA.[31–33] Importantly, it was shown that T cell epitopes common to pH1N1 and pre-pandemic HA stimulated memory CD4+ T cell responses; epitopes unique to pH1N1 HA failed to mount a significant CD4+ T cell response.[34] This could be one of various reasons that explain the poor antibody response to H7N9 influenza. Epitope predictive algorithms found 11 putative, broadly reactive CD4+ T cell epitopes, of which only four are conserved in seasonal influenza. This contrasts with 16 broadly reactive epitopes found in pH1-HA, of which 13 are conserved in pre-pandemic H1-HA.[29] Furthermore, an H7-HA peptide screen showed that only ~ 60% of persons never exposed to H7N9 influenza develop CD4+ T cell responses, primarily to sequences found in the HA2 domain in regions highly conserved with H3-HA.[34] While seasonal influenza elicits memory CD4+ T cells that cross-react with H7-HA epitopes, it appears to be insufficient to generate protective antibodies in H7N9 vaccination. Given the advantages memory CD4+ T cells offer – higher frequencies and lower thresholds for antigen stimulation and co-stimulation in comparison with naïve cells[35-37] – a vaccine strategy that potentiates memory CD4+ T cell recall may improve H7N9 vaccine responsiveness.

CD4+ T cell help is required for antibody development

Neutralizing antibodies are considered the best correlate of vaccine efficacy for the majority of licensed vaccines. Generation of antibody after vaccination depends on follicular helper T cells (Tfh), a subset of CD4+ T cells that are specialized for providing help to B cells to support class switch recombination, affinity maturation, and B cell differentiation into long-lived plasma cells and memory cells in germinal centers (GC) in secondary lymphoid tissue.[38,39] A signature Tfh marker, CXCR5, is upregulated on naïve CD4+ T cells after antigen stimulation, permitting localization to the B cell zones of lymphoid tissue.[40,41] Mature Tfh cells expressing PD-1, ICOS and Bcl-6 provide signals for B cell survival and differentiation via CXCL13, IL-4 and IL-21 production as well

as CD40L expression. In humans, where access to lymphoid tissue is not feasible, circulating Tfh cells are surrogates for understanding human immune responses to vaccination.[42] Like GC Tfh, circulating Tfh express CXCR5 and PD-1, secrete IL-21 and are able to promote B-cell differentiation in vitro.[43] Circulating ICOS+CXCR3+CXCR5+ Tfh are associated with protective antibody responses after seasonal influenza vaccination in young adults.[44,45] Thus, a vaccine strategy that boosts Tfh responses may improve H7N9 vaccine responsiveness by inducing CD4+ T cells.

HA-specific CD4+ T cells are linked to neutralizing antibody responses

CD4+ T cells exhibit a range of functions, including cytotoxicity and support for memory CD8+ T cell development, in addition to providing help to B cells for neutralizing antibody production. An H7N9 vaccine strategy that focuses the CD4+ T cell response on development of increased HI antibody titers would improve vaccine responsiveness. Recent studies have shown that antigen specificity of CD4+ T cells responding to influenza vaccination is linked to neutralizing antibody responses.[34,46] Mice that develop CD4+ T cell memory to HA exhibit enhanced neutralizing antibody levels and lower lung viral titers in comparison with mice bearing nucleoprotein-specific CD4+ T cell memory.[47] Linked specificity is also observed in humans who receive inactivated virus vaccine, which contains internal proteins in addition to the critical antigen HA. Monovalent pandemic H1N1 and H5N1 vaccination studies show that HI titers correlate with HA-specific CD4+ T cell expansion, but do not track with NP-specific CD4+ T cell levels.[34,46] Moreover, circulating Tfh in humans are predominantly HA-specific.[48] These studies suggest a vaccine strategy that boosts protective antibody responses via memory CD4+ T cell induction could rely on HA-derived epitopes, despite closer sequence identity between seasonal and avian influenza internal antigens.

HA has a high tolerance for mutations

Another rationale for introducing seasonal HA CD4+ T cell epitopes into H7N9 HA is the remarkable capacity for influenza HA to tolerate mutations without compromising its structural and essential functional properties. As many as 18 different HA subtypes have been described, suggesting that the protein structure can accommodate a wide variety of amino acid substitutions.[49] Notably, a recent study showed that when mutant viruses that incorporated ~ 10,000 single amino acid mutations to HA were generated, viruses that successfully replicated could accomodate mutations at any site in the protein other than those involved in receptor binding to host cells.[50] This extraordinary tolerance for mutation makes HA an ideal target for rationale design, where memory CD4+ T cell epitopes from seasonal HA are introduced into their corresponding locations in avian HA. Despite the relative low risk, care must be taken when making sequence modifications to avoid perturbation of the global HA fold and neutralizing epitopes that are needed to raise HI antibodies that recognize the wild type HA circulating in

nature. With the availability of molecular modeling and simulation methods, it is feasible to strike a balance between preserving structure and improving immunogenicity to rationally design vaccines for production and immunogenicity and efficacy testing. Indeed, this is akin to a strategy we and others use to address the challenge of biologic drug immunogenicity.[51–53] While the goal for biologics is to reduce anti-drug antibodies, in contrast with objective of vaccination, sequence modifications that delete CD4+ T cell epitopes have been successfully balanced with maintaining biologic structure and activity to improve drug safety and efficacy.

First generation immune engineered H7N9 HA

We designed a novel H7N9 HA vaccine that illustrates the immune engineering concept. Using immunoinformatic methods, we predicted H7-HA$_{297-309}$ to be a promiscuous CD4+ T cell epitope that may induce a regulatory T cell (Treg) response, which could be another reason for the delayed and weak HI responses in natural H7N9 influenza infection and in vaccine trials. We found that this sequence activates human CD4+CD25^{high}CD39+FoxP3+ Tregs and suppresses H7N9-specific effector (IFNγ+) T cell responses measured by ELISpot assay in naïve individuals.[54] To engineer an antigenically improved vaccine, we made three amino acid substitutions in H7-HA that introduced the corresponding sequence in H3-HA (H3-HA$_{306-318}$), a highly conserved and broadly reactive CD4+ T cell epitope. Structural modeling suggested that the H7-HA structure could accommodate these substitutions without destabilizing the protein, enabling introduction of this epitope to enhance immunogenicity. Not only have there been very large numbers of exposures to this epitope over many influenza seasons, but also > 95% of infected humans may have developed H3-HA$_{306-318}$-specific CD4+ T cell memory because the epitope binds all eight class II HLA supertype alleles.[55,56] Indeed, this epitope is commonly used as a positive control for influenza immunoreactivity in cellular assays. Therefore, a novel H7N9 HA vaccine bearing H3-HA$_{306-318}$ could preferentially recruit memory CD4+ T cells and simultaneously avoid Treg induction to increase antibody responses to this otherwise poorly immunogenic HA.

Characterization of the engineered rHA (H7-HA-Opt1) and comparison to the wild type rHA demonstrated both preserved antigenicity and improved immunogenicity in humanized mice.[57] Three monoclonal antibodies raised against wild type H7-HA recognized H7-HA-Opt1 with affinity equivalent to the wild type protein, suggesting that modifications did not induce significant structural perturbations. Similarly, human polyclonal sera demonstrated identical binding profiles against H7-HA-Opt1 and wild type H7-HA.[57] Importantly, immunizations of NOD/SCID/JAK3(null) immune-deficient mice reconstituted with human PBMCs (N = 8) using non-adjuvanted H7-HA-Opt1, stimulated a 5-fold greater anti-H7-HA IgG titer and 20-fold greater anti-H7-HA B cell frequency over mice immunized with wild type protein. Taken together, these experimental data provide evidence that CD4+ T cell epitopes significantly affect the immunogenicity of H7N9 influenza vaccines and should be taken into consideration in vaccine design.

Conclusions

H7N9 viruses, along with many other emerging influenza subtypes, demonstrate the necessity to develop faster, more efficacious vaccines against potential pandemics. Advances in immunoinformatic methods coupled with structure modeling allow us to create vaccines that enrich for immunogenic T cell epitopes as a strategy to enhance both the antibody and cellular responses against H7N9 antigens. In addition, immune engineering can be applied as a vaccine design strategy for other pathogens to optimize promising antigens that are intrinsically poorly immunogenic due to lack of $CD4^+$ effector T cell-inducing or presence of regulatory T cell-inducing epitopes. This strategy may prove to be critical against pathogens capable of rapid spread and causing high numbers of severe illness and death in an immunologically naïve population.

Disclosure of potential conflicts of interest

Coauthors ADG, WDM, LM, BB and CMB are employees of EpiVax, and two (ADG, WDM) are majority stockholders. These authors recognize the presence of a potential conflict of interest and affirm that the information represented in this paper is original and based on unbiased observations. NKY, HS, CS and TMR declare no competing interests..

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