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Generation of a broadly reactive influenza H1 antigen using a consensus HA sequence

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ABSTRACT

H1N1, one of the most prevalent influenza A virus subtypes affecting the human population, can cause infections varying from mild respiratory syndrome to severe pneumonia. The current H1N1 vaccine needs to be updated annually and does not protect against future outbreaks. Here, we downloaded 2,656 HA protein sequences of human H1N1 viruses from the NCBI influenza database (up to the date of Aug. 2012) and constructed a phylogenetic tree of these H1 proteins via the neighbor-joining method using MEGA 5.0 software. A consensus H1 protein (CH1) was generated and was further modified with published conserved T-cell and B-cell epitopes. Interestingly, this CH1 protein is genetically similar to an H1 isolate obtained during the 1980s (A/Memphis/7/1980), indicating that a universal HA antigen may exist in nature. Vaccination with a DNA vaccine expressing CH1 elicited broadly reactive T-cell and B-cell responses to heterologous H1N1 viruses, though this vaccine did not successfully neutralize pdm09 H1N1 viruses. A combination of CH1 and pdm09 HA in a DNA vaccination neutralized pdm09 H1N1 viruses and protected mice from lethal infections by all representative H1N1 viruses. Moreover, a recombinant chimeric PR8-CH1 virus carrying HA sequence of the consensus H1 and all other seven genes from the PR8 strain was highly attenuated in mice, with a lethal dose (LD_{50}) of more than 10^6 pfu. Vaccination with PR8-CH1 virus provided complete protection against infections by heterologous H1N1 strains. Taken together, a universal H1 antigen, CH1, was developed by constructing a consensus HA sequence, and the PR8-CH1 virus containing this consensus sequence elicited broadly protective immunity against heterologous H1N1 viruses.

1. Introduction

Influenza is an infectious disease caused by infection of the respiratory tract by influenza viruses. As segmented RNA viruses, influenza viruses, especially the major subtype of influenza A viruses, possess a large gene pool and can infect a wide range of host species. The influenza A virus strains circulating annually differ from those of the previous year; thus, the viruses can partially escape the pre-existing immunity in the population and cause seasonal influenza. Seasonal influenza epidemics are estimated to affect approximately 10% of the world population, causing symptoms ranging from mild infection to severe pneumonia and resulting in 3–5 million cases of severe illness and 250,000–500,000 deaths [1]. Occasionally, newly emerging influenza A viruses created by genetic re-assortment can cause pandemics and severe disease in humans because the public has little or no resistance to these new viruses. The most notorious influenza pandemic, the 1918 Spanish H1N1 pandemic, killed approximately 50 million people worldwide [2]. The other three major pandemics in human history are the 1957 Asian H2N2 pandemic, the 1968 Hong Kong H3N2 pandemic, and, most recently, the 2009 Mexico H1N1 pandemic (pdm09) [3]. In addition to influenza pandemics, direct transmission of influenza A viruses from avian to human has also caused great fear and concern due to outbreaks such as those of the avian H5N1 influenza A virus in Hong Kong in 1997 [4] and the avian H7N9 influenza A virus in China in 2013 [5]. Among all influenza A virus subtypes, H1N1 is the most prevalent subtype in the human population and has caused many outbreaks throughout history [6].

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Vaccination is the most effective prevention against influenza virus infection. However, due to antigenic drift and shift, the most widely used inactivated influenza vaccine needs to be updated and individuals re-inoculated annually. Although there is no cross-protection between different subtypes of influenza viruses in natural infections [7], increasing evidence suggests that one subtype of influenza virus may induce cross-reactive immune responses against other subtypes both in nature and in experimental infections [8–10]. Thus, the concept of a more robust "universal vaccine" was proposed. Ideally, a universal vaccine would protect against multiple subtypes of influenza A virus or against all virus strains within a certain subtype. The targets of universal vaccines are conserved viral proteins or conserved domains within viral proteins, such as HA2, M2e, M1 and NP [11]. Among these, the envelope glycoprotein hemagglutinin, or HA, is the main antigen present on the surface of the influenza virion and is responsible for receptor binding and membrane fusion [12]. HA is synthesized as a precursor and cleaved into two smaller polypeptides, the HA1 and HA2 subunits, which comprise the HA globular head and HA stalk domains, respectively [13]. The HA stalk domain, which is highly conserved among subtypes [14], is the target of universal vaccines in many research studies [11]. However, generation of replicating viruses harboring only the stalk domain is impossible; therefore, the use of the HA stalk antigen is limited to vaccine approaches using recombinant proteins, virus-like particles (VLPs), or DNA vaccines. A full-length HA protein containing all functional domains and consensus epitopes is urgently needed for rapid application in the current vaccine production system and to prevent future pandemics.

DNA vaccines are commonly used to develop universal influenza vaccines because they can induce both humoral and cellular immune responses. Since the first publication reporting the induction of cellular immune responses by a plasmid DNA encoding influenza NP protein in mice [15], many studies have conducted influenza DNA vaccination to induce cross-protective immunity. A plasmid expressing the HA protein of HK-H5N1, formulated by polyethylenimine and administered intranasally, induces the production of serum antibodies that are cross-reactive to a heterologous H5N1 influenza A virus [16]. In another case, a bivalent DNA vaccine encoding the chicken H5N1 HA and NP proteins induced both heterosubtypic humoral and cellular immunity to a chicken H9N2 virus [17]. To improve the immune response potency of DNA vaccines, efforts have been made to either increase antigen expression or to search for effective adjuvants. In a recent report, a plasmid encoding the H1N1 HA protein significantly improved mucosal and systemic immune responses when co-administered with DNA encoding the rhesus GM-CSF gene [18]. To improve gene expression, an H5 HA gene was codon optimized and inserted into a eukaryotic expression vector (pCAGGS), greatly increasing both the gene expression level and immune response of the antigen [19].

In the present study, we downloaded over 2,000 HA protein sequences of human H1N1 influenza viruses from the NCBI influenza database (up to Aug. 2012). These sequences were then subjected to multiple alignment and phylogenetic analyses with MEGA 5.0. A consensus sequence was designed accordingly. Meanwhile, we summarized conserved T-cell and B-cell epitopes in an H1N1 HA protein that had already been published. These epitopes were then used to replace their counterparts in the consensus sequence to obtain a final consensus sequence, CH1, and its reverse-translated DNA sequence, *CH1*-HA. When *CH1*-HA was inoculated as a DNA vaccine, it induced a broadly reactive T-cell response to H1N1 viruses from different phylogenetic subgroups, including the 2009 pandemic pdm09 H1N1 virus. However, antibodies induced by CH1 could only neutralize viruses from the 1940s and 1990s and not the pdm09 virus. The combination of DNAs expressing CH1 and HA from pdm09 elicited both T-cell and B-cell responses to the pdm09 virus and further protected mice from lethal infection of heterologous H1N1 viruses. Surprisingly, a 7:1 virus expressing the *CH1*-HA gene within a PR8 backbone was highly attenuated and protected against infections by heterologous H1N1 viruses, including pdm09 and pandemic-like viruses.

2. Results

2.1. Obtaining consensus H1 protein sequences by phylogenetic analysis

To obtain the consensus H1 protein, we performed a phylogenetic analysis by downloading all H1 protein sequences of human isolates from the NCBI influenza database (up to Aug. 2012). A total of 2,656 full-length sequences were retrieved after collapsing identical sequences, and the phylogenetic tree was constructed using the neighbor-joining method displayed in Fig. 1 in either a rectangle shape (Fig. 1A) or a radiate shape (Fig. 1B). It was noted that since the outbreak of pandemic H1N1 virus in 2009, the HA genes of pdm09 viruses have been abundantly sequenced. Thus, to obtain a consensus sequence presenting the highest antigenic similarity to all H1 proteins, the over-sequenced HA proteins from pdm09 were considered as a single subset. A vertical straight line crossing the root of the pdm09 branch was drawn on the rectangle tree, and each crossover point on the branches of the tree was considered as a different subset (Fig. 1A, framed). A consensus sequence of each subset was obtained by aligning all protein sequences within the individual subset using ClustalX software. A final consensus H1 sequence was obtained by further aligning subset consensus sequences. We also integrated all published T-cell and B-cell epitopes (Suppl. Table 1) into this consensus sequence, and the final sequence was named "CH1".

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2018.06.048.

When the phylogenetic tree was displayed in a radiate shape, sequences with high similarity clustered into three subgroups: Group 1, the latest strains of pdm09 viruses; Group 2, mainly ancient strains from 1933 to 1980; and Group 3, recent strains from 1995 to 2008 (Fig. 1B). CH1 was located in Group 2 among isolates from before the 1980s (Fig. 1B). Interestingly, CH1 showed high homology to the HA protein of the A/Memphis/7/1980(H1N1) isolate, and their sequences differed from each other by only three amino acids (Suppl. Table 2).

2.2. Expression of the consensus H1 in a plasmid and in a recombinant virus

To examine the expression of *CH1*-HA, the protein sequence was reverse translated into the corresponding DNA (with reference to the HA sequence of A/Memphis/7/1980(H1N1)) and cloned into the eukaryotic expression vector pCAGGS with a C-terminal FLAG-tag, and this construct, named "pCH1", was then transfected into 293T cells. The data in Fig. 2A show that *CH1*-HA was well expressed in mammalian cells. The *CH1*-HA gene was further cloned into the pHW2000 vector to generate a 7:1 recombinant virus expressing *CH1*-HA and all other seven viral genes from the PR8 influenza A virus strain (PR8 backbone). Such a 7:1 virus, named "PR8-CH1", can be used as a vaccine candidate in the current cell-culture or egg-based vaccine production systems. As shown in Fig. 2B, the PR8-CH1 virus formed plaques of a similar size to those of the wild-type PR8 virus on MDCK cells. PR8 and PR8-CH1 also exhibited similar growth kinetics, indicating sufficient propagation of PR8-CH1 in cell cultures (Fig. 2C).

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