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The Future of Influenza Vaccines: Developing Tools to Match Glycosylation Patterns Relevant for Protection

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Abstract

Influenza is a viral disease that is easily transmissible, and it is found around the world and can affect anyone regardless of their age group. There are 3 types of influenza viruses: A, B and C. Influenza viruses A and B are usually responsible for causing outbreaks of influenza from limited to major epidemics or even pandemics. The main preventive measure against this virus is annual vaccination, and the World Health Organization annually publishes recommendations for the production of influenza vaccines, but the protection observed so far has not been optimal. It has been proven that using egg as a substrate for vaccine production causes changes in the structure of proteins on the surface of influenza virus, and these changes could be involved in the low effectiveness of vaccines against influenza. Here we comment about platforms currently used to produce viruses for inclusion into influenza vaccines, and suggest alternatives to improve glycosylation patterns to resemble more closely those found in viruses infecting human beings, aiming to improve the effectiveness of protection conferred by these new vaccines.

Keywords: Influenza; Vaccine; Glycosylation; Substrates

Short Communication

Influenza is a viral disease that is easily transmissible, and it is found around the world and can affect anyone regardless of their age group. There are 3 types of influenza viruses: A, B and C. Influenza viruses A and B are usually responsible for causing outbreaks of influenza from limited to major epidemics or even pandemics, on the other hand, type C viruses cause mild symptoms and do not have great ability to cause an epidemic [1,2].

Since the last pandemic in 2009, the cases of influenza have not disappeared, despite the national prevention programs conducted in many countries. According to the World Health Organization (WHO), during the 2015-16 influenza period, in December, over 35,732 specimens were analyzed, where 89% of them were classified as influenza A and the remaining 11% was determined as influenza B. Out of the influenza virus classified as type A, 93.3% were influenza A(H1N1) and 6.7% was influenza A (H3N2) [3]. Almost half a year later, during the month of June, more than 55,586 specimens were analyzed and 60.1% were classified as influenza A and 39.9% as influenza B. From the viruses classified as influenza A, 86.2% were influenza A(H1N1) and 13.8% influenza A(H3N2) [4]. Although this year the number of cases of influenza has remained within the expected range, the possibility of an increase in the number of cases due to the circulation of new viral variants that arise of the influenza virus cannot be ruled out [5].

The main preventive measure against this virus is annual vaccination, the WHO annually publishes recommendations for the production of influenza vaccines, but the protection observed so far has not been optimal. In the 2015-16 influenza season in Europe, six circulating influenza virus H3N2 were poorly recognized by antiserum raised against egg-propagated influenza virus H3N2 A/Switzerland/9715293/2013, recommended for the vaccine in that season for northern hemisphere. The same 6H3N2 viruses had a somewhat better recognition by antiserum raised against egg-propagated H3N2 virus A/Hong Kong/4801/2014, which was already recommended for the preparation of the vaccine the following period 2016-17 influenza in the northern hemisphere [6]. This illustrates that one of the main causes of low effectiveness of vaccines against the disease, is the difference between the virus used in the vaccine and wild type viruses that transmit and provoke the disease.

Seven years since the last pandemic, the influenza virus with the highest incidence remains being the AH1N1 virus, even though this has been included as an antigen in all vaccines against influenza, either trivalent or quadrivalent [7]. This lead us to ask why this virus is still so incident, even more if it has not had substantial changes that warrant a change in the composition of current vaccines. The purpose of this work is to discuss the effect of the substrate used for production of vaccines, on the structural characteristics of influenza virus as well as to propose new alternatives to seek for improved vaccines against this disease.

Influenza vaccines are mostly produced in eggs and a small portion of them in cell substrates (Figure 1). It has been proven that using egg as a substrate for vaccine production causes changes in the structure of proteins on the surface of influenza virus, and these changes could be involved in the low effectiveness of vaccines against influenza [8,9].

Today, licensed vaccines are expected to induce in the patient, neutralizing antibodies that specifically bind to the hemagglutinin and neuraminidase, as well as antibodies capable of recognizing conserved protein regions to generate an adequate cellular immune response mediated by T cells [10]. Despite much effort, the protection conferred by available vaccines is limited to a certain percentage of the population where it was applied. Historically, it has been estimated that the major constraint is the high rate of mutability of influenza viruses

[16,17], as well as the possibility of recombination between different viral strains, the end result is the emergence of virus structurally different that escape immune mechanisms previously generated in individuals exposed to other viruses or vaccines [18].

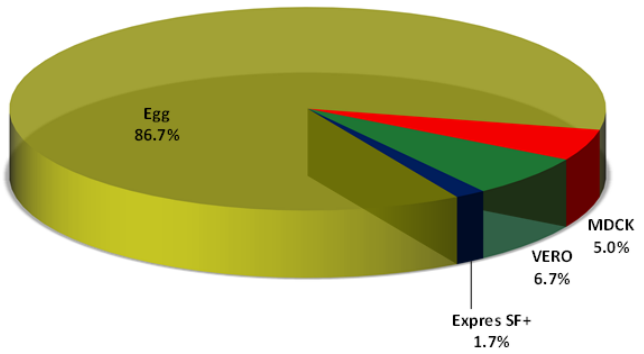

Figure 1: Substrates used in vaccine production. Distribution of vaccines currently licensed and produced in the following substrates: egg, MDCK cells derived from dog kidney, Vero cells derived from monkey kidney, as well as Express SF+ cells derived from insect cells, the chart was derived using data from 60 vaccines currently marketed [11-15].

The influenza virus AH1N1 used for the preparation of vaccines has not changed since the last pandemic of 2009 [7], mainly because no significant changes in the nucleotide sequence of the hemagglutinin (HA) has been reported [19]. HA is found on the surface of the virus [2], and it is the main viral protein used as antigen in vaccines. Nevertheless, the effectiveness of the vaccine has not been the one expected [20], therefore at present new alternatives or improvements to existing vaccines remain under investigation.

Major successes in research on influenza vaccines have helped to develop new technologies that have achieved the reduction of production time or increased the performance of the antigen [21]. To achieve this, various substrates and conditions for production of the antigen were developed [22-27], thus obtaining recombinant vaccines [28,29], virus-like particles [30,31] and viral-vectored vaccines [32] all of these strategies start from a characterized virus or nucleotide sequence, from which viral particles or proteins that constitute the vaccine antigen are generated.

However, and despite that antigens produced are immunogenic or even capable of inducing neutralizing antibodies following immunization, there is an important limitation, persistent in all these influenza vaccines production systems: post-translational modifications, which occur particularly in each host or expression system. The fundamental post-translational modification of hemagglutinin (HA) and neuraminidase (NA) occurs via glycosylation on several amino acid residues [33]. Glycosylation is an enzymatic process, controlled by the host cell glycosyltransferases, which operate in the Golgi apparatus during maturation of any glycoprotein [34]. Due to this process, the same viral sequence can generate different protein structures when expressed in different hosts. This leads us to consider that new vaccine production alternatives, would generate virus whose glycoproteins have the glycosylation pattern of: avian cells, insect cells, or mammal cells (Table 1) [8,35-37]. Table 1 show the effect of different kind of cells in the structure of the hemagglutinin of the influenza virus H5N1, where it is interesting to note that the glycans vary in complexity, in particular the human and mammalian cells show more branched and complex structure than the glycans generated in the chicken embryo or even in the insect cells that were previously glycoengineered to synthesize human-like N-glycans.

Source	Substrate	Glycans in the HA*	Reference
Hen	Embryonated egg	~8–9 monosaccharide units, neutral and highly branched	[8]
Insect *Spodoptera frugiperda*	*SfSWT-7* (Glycoengineered to synthesize human-like N-glycans [8])	6–9 monosaccharide units, Two additional N-glycosilation sites not presents in higher eukaryotic cells	[8, 36]
Human Embryonic Kidney	HEK293	~12 residues, complex glycans were highly sialylated (α2,3 and α2,6), and also were highly branched	[8, 34]
Chinese Hamster Ovary	CHO	8 to 18 residues, Complex glycans with higher sialylation with α2,3 linkage exclusively	[34, 36]
*The glycans of hemagglutinin were analyzed from H5N1 viruses, which were previously developed in substrates from different sources.			

Table 1: Influence of substrate in the glycosylation pattern of hemagglutinin.

Today, most vaccines are produced in chicken embryo (Figure 1), so that persons immunized with them are receiving an antigen whose glycoproteins show structural differences to those present in wild type virus circulating among human beings. Despite this situation, these vaccines have historically shown a moderate capacity in preventing influenza infections [38]. Current trends indicate that cell cultures will replace the embryo as a substrate for vaccine production [24,39]. It is too early to know whether these changes will positively impact the capacity to confer protection, but according to the structural vaccinology, it is a fact that the greater structural similarity between the antigen of a vaccine and that present in the pathogen that is transmitted between humans, the greater the protection induced, because the antibodies generated will have greater capacity of recognition for epitopes of the infectious agent [40], in influenza, the main antibodies that neutralize the infectiousness will be those able to bind to the HA [41]. HA is a glycoprotein that is responsible for the binding and penetration into the cell [42].

Considering the above mentioned, during the production of influenza vaccines, glycosylation conferred by the substrate will cause glycoproteins HA and NA to show differences to those in wild type virus. Previous studies have shown that differences in glycosylation have consequences directly related to the capacity of protection [42] and may even mask epitopes for neutralizing antibodies [43]. Besides, the virus derived from substrates phylogenetically closer to human ones, was able to induce antibodies with greater protection capacity and increased release of interleukin-2 involved in the protective immune response [44].

Furthermore, it has been observed that for proper cellular infection is necessary a glycosylation pattern that avoids steric hindrance with the target cell [42,43] and moreover allowing interaction of HA with its receptor, which is directly related to the virulence of the virus [2,45,46]. The viral neuraminidase also plays an important role in the elimination of certain glycans of viral receptors, so it cannot be ruled out that it could also modify those present in the HA allowing infectivity [47].

Recent efforts are focused on finding new substrates that allow the production of virus bearing more similarity to those transmitted between humans. Some studies have evaluated cell lines derived from human cells such as HEK293 [34,48], PER.C6 [34], Calu-3 [49] or lines such as SfSWT-7 derived from insect cells, the latter genetically modified to produce glycosylation patterns similar to humans [8]. The results obtained thus far have shown that the antigens generated could have greater similarity with the wild type virus transmitted among humans; however this remains to be confirmed, as currently it has not been possible to characterize the type of glycans present in the wild type virus. This is due to the fact that during the isolation of influenza virus from human samples, the glycosylation pattern will be inevitably altered when the viruses are replicated on the substrates of propagation. Efforts should be made in the short term to achieve concentrated and purified virus-positive samples, a complex procedure since most of the positive samples have a very low viral load as well as the possibility of having other respiratory viruses or microorganisms, which also present glycoproteins in their surface, which can potentially mask or interfere with the characterization of wild type influenza viruses. Moreover, one cannot rule out that the human species as a host of influenza virus could lead to differences in glycosylation patterns, as it has been seen that under pressure from the host, it is possible to select new glycosylation sites that change the antigenic structure, facilitating the avoidance of pre-existing immune response [50].

In the search for the ideal substrate for vaccine production, the possibility of generating it through genetic engineering is not ruled out. Current cell editing systems based on CRISPR/Cas9 may allow to quickly generating cells that synthesize viral antigens, structurally more similar or ideally identical to wild type influenza viruses. This technology has already been applied to obtain cells to represent various models of patients in interaction with the influenza virus [51]. The development of a substrate with the appropriate enzymatic machinery to generate identical glycoproteins to those produced by human cells would have an even greater scope, since there are biotechnological products, that even with the recombinant DNA technology, cannot be considered identical to humans, due to the type of post-translational modification present in existing expression systems [34].

Given the importance of the effects of glycosylation during the synthesis of viral glycoproteins, an increasing trend is to characterize the type of glycans added in the new substrate of production of influenza vaccines. Matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS) [8,52,53], several variants of HPLC [35, 36] and capillary gel electrophoresis with Laser-induced fluorescence detection (CGE-LIF) [35,37,54] are tools that greatly improve chances of determining such patterns more precisely.

As a general conclusion we can highlight that there are several studies focused in improving the effectiveness of AH1N1 vaccines. In fact, 3 areas or study are well recognized: (1) The first one is related to the viral antigen, in where the WHO Global Influenza Surveillance and Response System (GISRS), has had a main role for more than 60 years in the monitoring, characterization, and selection of viral antigens for vaccine production, in order to achieve a protective response against potential strains that could provoke epidemic outbreaks [55]; (2) the second field is focused on the substratum in where the antigen is produced, many studies are conducted so as to determine the feasibility and effectiveness of vaccines developed on cell cultures for example de VERO cells [56,57]; (3) the last one is focused in improving the immunogenicity of the antigen, by using different kind of adjuvants such as alum or those based on the squalene-containing emulsion MF59 [58] or the oil-in-water emulsion Adjuvant System AS03 [59].

The current trend in research on influenza vaccines is to achieve an acceptable platform able to generate in a short time and with high performance, an antigen that guarantees to confer to the patient an adequate immunity. Current technologies are allowing to generate increasingly complex modifications such that there are expectations of developing cells or systems with specific enzymatic machinery, achieving biotechnological products with a glycosylation identical to humans, where biosynthesis of better antigens for vaccines against influenza will be easily achieved.

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