HUMAN VACCINES & IMMUNOTHERAPEUTICS
2020, VOL. 16, NO. 8, 1746–1752
https://doi.org/10.1080/21645515.2019.1701912

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REVIEW

Quadrivalent cell culture influenza virus vaccine. Comparison to egg-derived vaccine

Alberto Pérez-Rubio[a], Julio Ancochea[b], and Jose María Eiros Bouza[c]

[a]Dirección Médica, Hospital Clínico Universitario de Valladolid, Valladolid, Castilla y León, Spain; [b]Neumology, Hospital Universitario de la Princesa, Madrid, Spain; [c]Microbiology, Hospital Universitario Rio Hortega, Valladolid, Spain

ABSTRACT

Influenza virus infections pose a serious public health problem and vaccination is the most effective public health intervention against them. The current manufacture of influenza vaccines in embryonated chicken eggs entails significant limitations. These limitations have been overcome by producing vaccines in cell culture, which allow a faster and more flexible response to potential pandemic threats. Given the impact of influenza B virus on disease burden, the availability of quadrivalent vaccines is useful for increasing the rate of protection from disease.

This paper analyzes the limitations of the current production of influenza vaccine in eggs and the advantages of vaccines developed in cell culture, as well as their safety, tolerability, efficacy and effectiveness. Additionally, we reflect on the contribution of new quadrivalent vaccines from cell culture as an alternative in seasonal vaccination campaigns against influenza.

ARTICLE HISTORY
Received 22 September 2019
Revised 19 November 2019
Accepted 1 December 2019

KEYWORDS
Influenza; quadrivalent; cell cultived

Introduction

Influenza virus infections are considered a serious public health problem during seasonal outbreaks, while representing a constant threat through the appearance of new, potentially highly virulent pandemic strains.[1,2] Vaccination is the most effective public health intervention for reducing the impact of seasonal influenza.[3] However, influenza vaccines must be produced "ad hoc" each season to correspond to the constantly changing antigenic characteristics of circulating influenza viruses.[4] In addition, the conventional methods of producing inactivated influenza vaccines in the allantoic cavity of embryonated chicken eggs, have important limitations.[1,2,5,6] First, this production method requires the availability of a large number of eggs in a short period of time, and poses an increased risk of contamination that requires the use of anti-infective agents.[7] This necessitates a prolonged process of planning and execution, which can cause an insufficient supply of vaccines if some batches do not meet quality standards.[1] Secondly, after identifying the strain, it takes a minimum of 4–6 months to produce the vaccines and in the event that a pandemic strain appears and disseminates, there might not be enough time available for production or necessary quantities of eggs. Finally, the effectiveness of the annual influenza vaccine has been low in some recent years, especially for the H3N2 component, and has become a concern for global public health.[8,9] An important cause of the waning effectiveness has been attributed to the egg-based vaccine production process, since the mode of receptor-binding and domain binding-specificity are also modified to adapt to avian viral receptors during egg passage. Nevertheless, the structural and biophysical mechanisms involved in changes in antigenicity and the practical consequences for vaccine effectiveness resulting from adaptive substitutions in H3N2 viruses have not yet been fully explored.[10]

It seems pertinent to develop innovative techniques and procedures for the development of new influenza vaccines: production methods based on cell cultures, recombinant vaccines, vaccines based on reverse genetics, as well as live attenuated vaccines or live vector vaccines that have shown great potential in clinical trials.[11,12]

In recent years, new influenza vaccines have been licensed that employ mammalian cell lines in their production. These cell lines confer several advantages for the large-scale vaccine manufacture, including the ability to rapidly expand production in both a prepandemic and pandemic environment.[12-15] This vaccine production system offers more flexibility than egg-based systems, due to adequate substrate availability for viral growth and higher viral yields,[5] greater antigenic stability of hemagglutinin (HA),[16] and higher immune responses elicited than those produced by egg-derived vaccines.[17] In addition, unlike conventionally manufactured vaccines, cell culture-derived influenza vaccine (CCIV) can be produced in large quantities in a shorter period of time.[18] The manufacturing process of CCIV poses a lower risk of contamination, and does not require thimerosal, antibiotics or formaldehyde. As the production process of CCIV is egg-free, CCIV are suitable for people with any type of hypersensitivity or allergy to the egg proteins.[14] Moreover, cell culture facilities can be used for the production of other vaccines when they are not used for the production of influenza vaccine for prolonged periods.[5]

Particularly in the last decade, discrepancies have arisen between the circulating B strain and the recommended strain,

CONTACT Alberto Perez-Rubio albertoprz@gmail.com Dirección Médica, Hospital Clínico Universitario de Valladolid, Calle Ramón y Cajal 3, 47003 Valladolid, Castilla y León, Spain

which have reduced the effectiveness of trivalent vaccines (TIV)[4,19,20] and, increasingly, both B strains circulate in codominance in a given season.[4,19-21] However, predicting exactly which of the two lineages will be prevalent is difficult and the antigenic divergences between the two lineages of influenza B viruses are so important that they reduce cross-reactivity.[22] Different studies have analyzed the divergence of the vaccine strain from the wildtype, placing it at 42% in a Finnish study[23] covering 12 influenza seasons (1999–2012), and 50% in a Spanish study.[24] Given the significant impact of influenza B virus on disease burden, with an average of 24% to 30% of all influenza cases,[4,24,25] the poor accuracy for forecasting the predominant B virus strain, and the consequent compromise in immunity, the recently commercialized quadrivalent vaccines, with the addition of a second B strain to the trivalent vaccine, are very useful for increasing the rate of protection from protection.[5,26] Their benefits also potentially increase because they may not only reduce influenza cases, but also generate substantial savings,[19,27-29] which caused their inclusion in the recommended composition of seasonal influenza vaccine[30] and their use in certain risk groups.[31]

This article analyzes the advantages of cell-culture vaccines, their efficacy, effectiveness, safety and tolerability, and reflects on the contribution of new quadrivalent vaccines as an alternative in seasonal vaccination campaigns against influenza.

Effectiveness of egg-based vaccines

The first commercial influenza vaccines approved in the US were developed more than 70 years ago,[32] and although the annual manufacturing capacity is estimated at 400 million doses of trivalent influenza vaccine,[33] vaccinated persons are not immunized as fully as desired, and complete and comprehensive protection is still challenging.[9]

Conventional influenza vaccines confer substantially varying protection according to viral types and subtypes.[9] In the last decade, the effectiveness of the seasonal vaccine against H3N2 viruses has been particularly low.[9] While the vaccine's effectiveness was estimated at 67% for seasonal H1N1 (prior to 2009), 73% for H1N1pdm09 and 54% for type B, it was only 33% for H3N2 viruses.[9,34,35] The estimated effectiveness of the influenza vaccine for the 2017–2018 season in the USA was 40%.[36] A similar study carried out in Spain for the 2013/14–2014/15 seasons revealed an overall effectiveness of 36%.[37]

These results concerning the effectiveness of the influenza vaccine against circulating H3N2 strains compared to other influenza viruses is partly explained by the lack of antigenic agreement between circulating strains and the vaccine strain.[9] Antigenic drift can cause a substantial reduction in vaccine effectiveness. The authors of a study conducted by the *US Flu VE Network* found that the effectiveness against H3N2 in the 2014–2015 season was negligible for viruses of the genetic group of hemagglutinin 3C.2a that deviated antigenically, and 44% for 3C.3b viruses that were antigenically similar to the vaccine strain.[38] The manufacturing process of the vaccine may contribute to a low effectiveness against H3N2 by generating mutations in hemagglutinin induced by egg culture that affect its antigenicity.[39]

The recognition and neutralization of the influenza virus by the immune system has been the subject of extensive research due to its profound implications for vaccine design. The majority of human antibodies against the influenza virus are directed to the domain of the globular head of the glycoprotein hemagglutinin (HA). In H3N2 viruses, the main targets are five A-E antigenic domains.[40-42] However, most of the domain of the globular head has an intrinsically high mutational tolerance[43,44] that facilitates the escape from the immune system. The receptor-binding site (RBS) is conserved, but can still accommodate some level of mutation to evade antibody recognition.[10] Influenza virus often mutates to adapt to culture in embryonated chicken eggs, which may influence antigenicity and, therefore, the effectiveness of the vaccine.[10]

Hemagglutinin (HA) glycoprotein substitutions that often arise during serial viral culture change their antigenicity.[10] In this sense, the effect of a prevalent substitution, L194P, on H3N2 viruses obtained by growing them in the egg has been characterized. X-ray analysis revealed that this substitution increased the mobility of the 190-helix and its neighboring regions at antigen site B, which constitutes part of the RBS site and is immunodominant in recent human H3N2 isolates. Importantly, substitution of L194P decreased antibody-binding and neutralization by three orders of magnitude and significantly diminished the binding of human serum antibodies, i.e. antigenicity of HA.[10] The change in the receptor-binding mode associated with the L194P substitution provides an explanation of its ability to grow successfully in eggs.[10]

Although eggs provide a cost-effective way to grow influenza viruses, the abundance of avian type receptors in the chorioallantoid membrane[45,46] favors selection of variants that increase binding to avian type receptors (NeuAcα23Gal), and reduce the binding to human-type receptors (NeuAcα2-6Gal),[10,45,47,48] explaining the low effectiveness of the vaccine against H3N2.[45,46] These adaptive egg substitutions in HA negatively affect antigenicity,[49-54] which leads to a decrease in vaccine effectiveness.[10,53] In addition, genetic comparisons of hemagglutinin (HA) sequences of several egg-grown viruses similar to strain A, have revealed that substitutions in the amino acid sequence of HA alter their antigenic properties.[55]

Effectiveness of cell culture-produced vaccines

The immunogenicity and safety of influenza vaccines produced in cell culture have been extensively studied[2,14,21,56,57] as we describe in Table 1. Likewise, the immunogenicity and safety of the quadrivalent vaccine developed in cell culture (cQIV) have been evaluated, demonstrating not only non-inferiority compared to the trivalent vaccine,[58] but also the superiority for both influenza B lineages when comparing the geometric mean titer and seroconversion rates three weeks after the last

Table 1. Comparative studies on the immunogenicity, safety and effectiveness of cell culture vaccine versus egg-derived vaccine.

Author	Objective	Population	Results/conclusion
Ambrozatis[14], Vaccine 2009	Tolerability and immunogenicity	n = 1,200 (18–60 years)	Local reaction 27–31% CCIV vs 25% TIV Systemic reaction: overall CCIV 24–26% vs. TIV 23% Immune responses were similar for CCIV vs. TIV
Bart[21], Human Vaccine & Immunotherapeutics 2016	Immunogenicity and safety	N = 2,680	Overall antibody responses were similar in cQIV and TIV At day 22, the GMT and the percentage of subjects with seroconversion for unmatched B strains were higher in QIV than TIV.
Frey[56], Clinical Infectious Diseases 2010	Immunogenicity, efficacy and safety	n = 11,404 (18–49 years)	Baseline seroprotection rates, seroconversion rates, and antihemagglutinin GMT did not differ between CCIV and TIV. The overall vaccine efficacy was 83.8% (one-sided 97.5% CI lower limit, 61.0%) for the CCIV and 78.4% (one-sided 97.5% CI lower limit, 52.1%) for the TIV group. An A/H1N1 virus was isolated from 56 of 60 cases, including 43 in the placebo group, five in the CCIV group (vaccine efficacy, 88.2%), and 8 in the TIV group (vaccine efficacy, 80.3%). Three cases were caused by vaccine-like H3N2 strains and only one by a vaccine-like B strain. The percentage of study participants reporting solicited reactions was similar in each group.
Szymczakiewich[57], Journal of Infectious Diseases 2009	Immunogenicity and safety	18–60 years, n = 1,300; elderly persons > or = 61 years, n = 1,354	The immunogenicity of CCIV was non-inferior to that of the conventional vaccine for all three vaccine strains in both age groups, regardless of underlying health status. Both vaccines fulfilled European Union registration criteria and were well tolerated, with similar incidences of solicited local and systemic reactions in both age groups; the only significant difference was an increased frequency of mild or moderate pain with CCIV than the conventional vaccine among adult (22% vs 17%; $P < .05$) and elderly (9% vs 5%; $P < .001$) vaccines.
Hartvickson[59], International Journal of Infectious Disease 2015	Immunogenicity and safety	N = 2,333	cQIV met the non-inferiority criteria against all four vaccine strains and demonstrated superiority for both influenza B strains Similar percentages of subjects experienced solicited and unsolicited adverse events (AEs) across all groups
Barta[60], Human Vaccine& Immunotherapeutics 2016	Immunogenicity and safety	N = 2,680	QIV non-inferiority criteria for all vaccine strains and demonstrated superiority for both influenza B strains Between 48–52% subjects experienced≥one solicited AE. Serius AE were reported < 1%
Izureta[65], Journal of Infectious Diseases 2018	Relative vaccine effectiveness (RVE)	>13 million beneficiaries	Relative vaccine effectiveness for cell-cultured vaccine relative to egg-based quadrivalent vaccine was 10%
Szymczakiewich[66], Human Vaccine& Immunotherapeutics 2012	Immunogenicity and safety	n = 2,609	The safety profile of both vaccines was similar, no serious adverse events related to either vaccine occurred. Mild or moderate pain was the most commonly reported reaction. Reactogenicity was slightly higher in elderly subjects receiving CCIV/TIV concomitantly with PV [46% vs 37%; p = non-significant]. Both vaccines met CHMP licensure criteria for adults and elderly subjects. With concomitant CCIV and PV, all three CHMP criteria were met for A/H1N1 and A/H3N2, whereas the B strain only met seroprotection and GMR criteria.

vaccination.[59] The cQIV vaccine elicits strong immune responses against the four vaccine strains without signs of immune interference by the addition of a second strain of influenza B. The immunogenicity of cQIV and trivalent influenza vaccine from cell culture was comparable in both young and older adults.[60]

Producing vaccine with viruses grown in mammalian cells (for example, Madin-Darby canine kidney [MDCK] cells) prevents glycosylation introduced in the egg adaptation stage[54] and the substitution of HA L194P in subtype H3N2.[10] Early reports suggest that the effectiveness of cell culture-produced vaccine exceeds that of similar egg-based vaccines. Publicly available data from the Worldwide Influenza Center in London revealed that circulating H3N2 isolates from the Northern hemisphere influenza seasons from 2011–12 to 2017–18 had, in all seasons, a higher degree of antigenic similarity with MDCK-propagated reference vaccine than with egg-based reference vaccine strains. In half of the seasons evaluated, little or no antigenic similarity was documented between circulating viruses and the seed virus of the egg-based vaccine.[27] These data suggest that mismatch has occurred consistently with the H3N2 reference viruses propagated in eggs, more so than with reference viruses propagated in MDCK cells.[27]

In a trial comparing the efficacy of cell culture-derived influenza vaccine (CCIV) and the egg-derived inactivated trivalent vaccine (TIV) with placebo against laboratory-confirmed influenza in healthy adults in the United States, Finland and Poland during the 2007–2008 influenza season, the efficacy of CCIV was superior to that of TIV, although the differences are not statistically significant: CCIV showed an efficacy of 83.8% [61.0%–97.5%] against the vaccine strains and 69.5% [55%–97.5%] against circulating virus strains, whereas TIV showed an efficacy of 78.4% [52.1%–97.5%] against vaccine strains and 63.0% [46.7%–97.5%] against all wild-type virus strains.[56]

Not only the virological evidence supports a greater effectiveness for cell culture vaccines compared to those produced in eggs.[61-64] A recent study, based on the analysis of the 2017–18 influenza season dominated by A(H3N2) in a vaccinated population over 65 years of age, showed that the quadrivalent influenza vaccine (cQIV) was 11% (95% CI: 8%–14%) more effective in preventing hospitalizations and visits to the influenza clinic than comparable egg-based quadrivalent standard-dose

products.[65] The results indicate that cell culture and high-dose vaccines were significantly more effective in preventing hospitalizations and primary care visits due to flu than quadrivalent and trivalent egg-based vaccines.[65]

Another retrospective cohort study estimated the effectiveness of cQIV versus egg-based QIV (eQIV) for influenza-like respiratory diseases by analyzing electronic records or vaccination information systems for US primary care between August 1, 2017 and March 31, 2018. This study demonstrated that cQIV was statistically more effective than eQIV in the prevention of influenza-like respiratory infections as measured in primary care visits in the 2017/2018 influenza season. The relative effectiveness of cQIV was 36.2% while that of eQIV was 26.8% for the group of adults aged 18 to 64, both statistically significant estimates. The lack of statistical significance in the extremes of age precludes definitive conclusions about the relative effectiveness of cQIV in these age groups, mainly due to the small number of cases in pediatrics (4–17 years) or in adults over 65 years of age.[36]

Safety and tolerability of cell culture-produced quadrivalent vaccines

All vaccines developed in cell culture, both trivalent and quadrivalent, have been well tolerated and have had excellent safety profiles that make them ideal for influenza vaccination campaigns.[14,19,48,56,57,59,66]

Localized reactions were reported in 27% to 31% of subjects who received CCIV compared to 25% of those who received TIV, mostly erythema and pain. Localized reactions, including pain at the injection site, were mild to moderate; serious local reactions rarely occurred (≤1% of subjects in any vaccine group) and disappeared without sequelae. All vaccines induced similar rates of systemic reactions (24–26% after CCIV versus 23% after TIV) and the most common in any group were headache, malaise and fatigue. Similar to the localized reactions, the reported systemic reactions were mostly mild (1 to 10%) or moderate (<1 to 4%) and transient. Statistical differences have not been documented for any localized or systemic reaction, as well as unexpected adverse events (AEs), during the follow-up period of 3 weeks to 6 months, nor were other indicators of reactogenicity reported.[14]

In general, current studies show that revaccination with CCIV and TIV in adults or the elderly was equally well tolerated, with similar reactogenicity profiles for each age group. Safety, immunogenicity and reactogenicity were not affected by the type of vaccine received in previous influenza seasons, although reactogenicity rates are expected to increase with simultaneous administration of pneumococcal vaccine. This increase in reactogenicity observed in concomitant administration with pneumococcal vaccine is expected to resemble that reported in similar studies.[67,68] Concomitant administration had no impact on the severity of the AEs observed, and did not condition the antibody response to influenza antigens.

cQIV vaccines are presented as well tolerated, with a safety profile similar to that of TIV vaccines. The majority of the elicited responses were mild to moderate and transient, without serious AEs related to vaccination.[19,21,59]

In children under 18 years of age, vaccination with cQIV did not cause serious adverse reactions related to the vaccine or deaths. The reported AEs were generally mild to moderate in severity and limited to a duration of less than seven days. The most frequently reported local AEs were increased sensitivity and pain at the injection site. The most frequently reported systemic AEs were drowsiness, fatigue and headache. The body temperature of most subjects was within the normal range after the vaccination.[59]

According to Bart et al.,[21] in those over 18 years of age, the most commonly elicited AE was pain at the injection site, whose overall incidence was 33.6% in the cQIV group (33.6%) versus 27.8% and 29.4% in trivalent vaccine groups (cTIV1 and cTIV2, respectively). Severe pain was reported in 0.2% of subjects in the cQIV group and in 0.1% of subjects in the cTIV1 group. Rates from other local AEs were similar between the vaccine groups. The most commonly reported systemic AEs were fatigue and headache. Severe systemic AE were reported by <1% of the subjects.

Conclusions

Quadrivalent influenza vaccines from cell culture present an alternative for seasonal influenza vaccination campaigns. Results have proven their efficacy, safety and tolerability, and seem to support greater effectiveness, backed by greater antigenic stability of cell culture-derived vaccines, although more studies will be necessary to confirm these observations. Although the cost of production is higher and efficiency studies will be necessary to really determine the value of the vaccine, new techniques for influenza vaccine production are urged in a WHO strategy. The race toward the production of new influenza vaccines using new techniques, which began decades ago, has begun to bear fruits in recent years, and vaccines developed in cell culture will consolidate their use in influenza campaigns and increase their share in the global production of influenza vaccines.

In conclusion, cell culture-derived vaccines overcome the limitations of current egg-based vaccines, and can generate greater confidence in influenza vaccination, especially among health workers themselves, which allows for an enhanced uptake and better results of influenza vaccination.

Acknowledgments

The authors thank the support made by Seqirus for the preparation of this work.

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