Recent Progress of Influenza Vaccine Production

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Abstract: In today's world of relatively complete public health, people still struggle with flu epidemics. Influenza vaccine is a good means of protection against influenza. However, there are still some shortcomings in today's influenza vaccine production technology. This paper focuses on influenza vaccine production. It first introduces the characteristics of influenza virus and the importance of vaccination. Then it summarizes two common production methods: the traditional chicken embryo preparation and cell substrate culture taking Madin-Darby Canine Kidney Cells (MDCK) cells as an example). The chicken embryo method has a long history, but is limited by low production efficiency and potential antigenicity changes. MDCK cell-based production has advantages like cell receptors similar to human cells, yet faces challenges such as poor cell growth and safety concerns. By comparing these methods, the study aims to identify existing problems. The outlook suggests further research on mammalian cell cultivation and gene--editing to improve vaccine production, with the ultimate goal of combining big data and artificial intelligence (AI) to predict virus mutations and enhance vaccine efficiency.

1 INTRODUCTION

Influenza virus belongs to the family Orthomyxoviridae and is a single-stranded, negative-stranded RNA virus whose structure includes a core and an envelope. The core consists of nucleoprotein and RNA polymerase. The nucleoprotein is type-specific, and influenza viruses can be categorized into four types: A, B, C, and D. The envelope contains two important glycoproteins, hemagglutinin (HA) and neuraminidase (NA). The envelope contains two important glycoprotein spines, hemagglutinin (HA) and neuraminidase (NA). HA binds to host cell surface receptors and mediates viral entry into the cell, while NA contributes to the release and diffusion of newly formed viruses from infected cells, and both glycoproteins are susceptible to mutation. Influenza viruses are highly contagious, mainly through droplet transmission, but also indirectly through contact with contaminated hands and daily utensils. The average person is susceptible and usually develops the disease 1-4 days after infection. Symptoms include high fever, headache, malaise, muscle aches, cough, etc. In severe cases, the disease can lead to pneumonia, respiratory failure, and other serious illnesses. In severe cases, it can lead to complications such as pneumonia and respiratory failure, and even be life-threatening. Influenza viruses mutate easily. The HA and NA of influenza A viruses often mutate, leading to the emergence of new subtypes and pandemics. Influenza B viruses also undergo antigenic mutation, but to a lesser extent, usually causing localized epidemics. Influenza C viruses are generally disseminated and relatively mild. Influenza D viruses infect mainly pigs and cattle and are less pathogenic to humans. Despite significant improvements in healthcare and public health, viral infections remain one of the leading causes of human and animal disease worldwide. There is no doubt that influenza poses a considerable risk and potential threat to human health. Influenza is transmitted by airborne droplets, is labile and highly contagious, and most people are susceptible to infection (Jin et al., 2024). For example, the influenza A virus poses a global and ongoing threat to human health, causing between 290,000 and 600,000 deaths and as many as 5 million cases of severe illness each year (Sekiya et al. 2021). Although people nowadays pay more attention to public health and prevent influenza by wearing masks and other behaviors, this only prevents mass transmission and infection but does not reduce the likelihood of individuals being infected. It has been reported that the use of masks etc. does not reduce the risk of influenza, confirmed viral respiratory infections, influenza-like illness, or any clinical respiratory infection. However, in the case of influenza vaccination, the body's immune system is

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effectively activated, which can protect an individual and also control the mass spread of influenza. Therefore, influenza vaccination is a relatively economical and convenient way of control (Aimi et al, 1990). Influenza vaccines are made from artificially cultured influenza viruses that have been inactivated and then processed, with the main components being proteins such as the HA proteins described above. The traditional method of preparing influenza vaccines on a large scale is the production of chicken embryos. However, in the face of a global pandemic of highly pathogenic influenza, especially avian influenza, this method does not meet market demand. Therefore, research organizations and vaccine companies are collaborating to develop new production technologies such as cell culture. Compared with the production of influenza vaccine from chicken embryos, cell production of influenza vaccine has the advantages of high production efficiency and good results. In this paper, we summarize two common large-scale production methods, compare them, and analyze their advantages and disadvantages.

2 MECHANISM OF ACTION OFINFLUENZA VACCINE

Influenza vaccines can induce the body to produce immune and cell-mediated immune responses. The humoral response is mainly based on the systemic response IgG and the local antibody response sIgA, and the cellular immune response is primarily based on the T-cell response. Both responses are capable of inducing a cross-protective effect. Among them, IgA mainly prevents the influenza virus from being absorbed by the body, IgG mainly prevents the lower respiratory tract from being infected with the virus, and T cells mainly prevent further expansion of the viral hazard by specifically recognizing and removing the virus from infected tissues.

3 INFLUENZA VACCINE PRODUCTION METHODS

The chicken embryo method of vaccine preparation began in 1937 as the first successful method of growing influenza viruses. In 1941, the U.S. government approved the use of a vaccine prepared by this method, and in 1945 it was used on a large scale by the U.S. military, where it proved to be effective.

This crude vaccine was usually prepared by injecting the virus into the urocystic blastocysts of chicken embryos, culturing them for some time, and then passing them through erythrocytes and a series of relatively crude isolations, followed by formaldehyde inactivation of the virus to obtain the inactivated influenza virus or the crude vaccine.

In the 1960s, thanks to the rapid development and application of isolation techniques, people were able to better purify the virus, and the whole virus vaccine was born. Today, the commercially available chicken embryo influenza vaccine is highly effective and protects 90% of those vaccinated. (Belshe et al, 2001, Belshe et al, 2004, Belshe et al, 2004, Nichol, 2003)

3.1 Inactivated Influenza Whole Virus Vaccine

The influenza virus was injected into the allantoic fluid of chicken embryos and incubated until stable. After stabilization, the chicken embryo allantoic fluid was removed and inactivated with formalin. After passing the sterility test and inactivation test, the inactivated allantoic fluid is separated by ultracentrifugation or chromatography, and packaged and reprocessed to obtain the vaccine. The whole virus-inactivated vaccine obtained by this method has high side effects, does not apply to children under 6 years of age, and has a relatively narrow application range.

3.2 Influenza Virus Lysate Vaccine

Based on the whole-virus inactivated vaccine, the inactivated virus is lysed by selecting a suitable lysing agent, and only the biomolecules that can be recognized by the immune system are retained, such as HA (hemagglutinin protein), NA (neuraminidase protein), and part of the M proteins (divided into two kinds, M1 and M2, M1 is responsible for the assembly of the virus and budding, and M2 is responsible for the opening of the cellular ion channel to make the virus survive in the cell, M1 is responsible for the viral M1 is responsible for virus assembly and budding, and M2 is responsible for opening cellular ion channels to make the intracellular environment acidic, which leads to the fusion of the viral envelope with the endosomal membrane. The fusion of the envelope with the endosomal membrane facilitates the release of RNP (ribonucleoprotein: wrapped around the genetic RNA of the virus) and NP (nucleoprotein: tightly bound to the viral RNA to form RNP) proteins from the virus. This adaptation is relatively widespread (Luo

2012.)3.3 Subunit inactivated influenza vaccines

Based on the lysed influenza virus vaccine, HA, NA, and other proteins are broken down and purified by appropriate lysis conditions to form a vaccine, which can be adapted to a larger proportion of the population than previous methods. This method can be adapted to a larger proportion of the population, especially children and the elderly with weakened immunity, than previous methods.

4 CELL MATRIX CULTURE

Currently, MDCK, PER.C6, AGE.CR and EB14/EB66 cells have been established and used for influenza vaccine production (Wen et al, 2015, Chu et al, 2009, van et al, 2011). Among these cell lines, the MDCK cell line is currently more mature. Therefore, in this paper, MDCK cells are used as an example of cell-matrix culture.

4.1 MDCK Cell Production

Due to the evolution of the virus, vaccines produced from chicken embryos do not completely prevent influenza (this is due to some differences between the receptors on chicken embryo cells and those n the 2 mammalian cells), so the World Health Organization proposed the use of mammalian cells for vaccine production in 1995. Among them, MDCK cells were experimentally tested to be the most suitable for vaccine production (Huang et al, 2015, Suderman et al, 2021). MDCK cells, known as Madin-Darby Canine Kidney Cells, were first established by SH Madin and NB Darby of the Naval Research Laboratory at the University of California, Berkeley, USA, and were derived from the kidneys of healthy Cocker Spaniel dogs. This cell surface receptor is more similar to human cell surface receptors, so influenza viruses do not need to adapt to changes in cell receptors in culture, reducing the likelihood of mutation.

4.2 Advantages and Challenges of Using MDCK Cells for Vaccine Production

The receptors on the surface of MDCK cells are similar to those of human somatic cells, which can prevent viruses from mutating during the culture process. However, it faces many problems in the culture process, such as slow growth, high differentiation, low susceptibility to infection, and poor stability. In addition, it needs to be attached to the surface of the carrier in the culture medium for growth, which is easily limited by the surface area of the substrate and difficult to realize large-scale production. Therefore, it needs to be domesticated during the production process to facilitate its mass production.

4.3 Preparation of Vaccines from MDCK Cells

4.3.1 Microcarrier Culture of MDCK Cells

In 1967, Van Wezel (van,1967) introduced the microcarrier culture method so that the traditional method was no longer a limitation. Microcarriers are usually magnetic beads with a diameter of 90-350 μm and a density slightly greater than that of water (Alfred et al. 2011). It can support cell attachment growth with a larger surface area to volume ratio compared to single cells. Since microcarrier-based cell culture can be performed in suspension mode, metabolites, dissolved oxygen, nutrients, and pH of the medium can be controlled in a timely and efficient manner; moreover, cells cultured on microcarriers can better maintain their cellular phenotype because of the mechanical support provided to them by the microcarriers during the culture process (Healthcare, G. E et al ,2005). Some researchers compared the growth of an influenza virus strain (A/PR8/34) in MDCK cells (Tree et al, 2001) and compared the viral yields of different porous and solid microcarrier cultures, as well as those of conventional cell culture methods and chicken embryo cultures. The higher number of cells in solid vector culture may be because solid vectors have a more suitable surface for MDCK cell attachment, while porous vectors may have other limitations such as oxygen and nutrient transport limitations, and accumulation of wastes within the structure, in addition to differences in the concentration of solid and porous vectors per milliliter of liquid and the speed of agitation that may also affect the growth of MDCK cells. This effect of different microcarriers in cell culture has been demonstrated in other cell lines such as BHK cells (Alves et al, 1996).

4.3.2 MDCK Cell Suspension Culture Domestication Technology

This method mainly solves two problems in MDCK cell culture, one is to solve the walling problem in MDCK cell culture, and the other is to solve the serum-dependent problem in MDCK cell culture. At

present, there are two main ways to domesticate MDCK suspension cells: (1) Before converting MDCK cells into suspension cells, they are first adapted to be cultured in a serum-free medium of some known composition. This process can be accomplished by both direct and indirect methods. The direct method is a complete media change. Specifically, the direct method of domesticating MDCK cells is the gradual conversion of traditional serum-rich medium to serum-free medium. This process can be carried out in steps, gradually reducing the proportion of serum while increasing the proportion of serum-free medium, so that cultured MDCK cells can gradually adapt to the serum-free environment and grow stably. An indirect way to domesticate MDCK cells is to introduce specific small molecule compounds or biofactors during the domestication process to facilitate the adaptation of the cells to serum-free culture conditions. These biokines and compounds mimic growth factors and cell signaling pathways found in serum and help MDCK cells adapt to the serum-free environment. The goal of these domestication methods is to reduce or eliminate the serum dependence of MDCK cells and adapt them to serum-free culture conditions. This will fulfill biosafety requirements, reduce the potential risk of exogenous pathogenic factors, and lower production costs. In conclusion, the domestication methods of MDCK suspension cells mainly include direct and indirect methods, which are cultured under serum-free culture conditions to gradually reduce the dependence on serum and provide more applicable cell lines for further research and production. (2) Gene Modification. It has been documented that the expression of the full-length human Siat7e gene correlates with the wall-dependence of the cells. The Siat7e gene encodes the human sialyltransferase ST6GalNAc V, which is responsible for the synthesis of GD1α (glycosidic aminoglycoside) from GM1b (monosialylguanoside), and shows higher levels of Siat7e gene expression in non-adherent-dependent HeLa cells as compared to adherent-dependent HeLa cells (Jaluria et al ,2007), and when inhibition of Siat7e gene expression was used with SiRNA technology, significantly enhanced adhesion properties were observed in these cells. Siat7e gene expression and a significant enhancement of the adhesion properties of these cells were observed when Siat7e gene expression was inhibited using siRNA technology. This implies that the Siat7e gene may have an inhibitory role in the cell wall attachment process. This finding made it possible to make mammalian cells grow without wall adherence by genetic modification. Chu et al (Chu et al ,2009) constructed MDCK cell lines stably expressing Siat7e by altering the adherence-dependence of MDCK cells through a genetic engineering approach so that they could be cultured in suspension; they utilized human Siat7e genes to express Siat7e genes in suspended MDCK cells. They transformed the eukaryotic expression plasmid of the full-length human Siat7e gene into E. coli DH5α, purified and extracted the plasmid ST6GalNac V for transfection by the QIAprep Spin Miniprep kit (Qiagen), and then transfected the MDCK cells with the transfection reagent Lipofectamine2000 Regent to express the Siat7e in MDCK cell lines. MDCK cell lines expressing Siat7e were altered to be non-adhesion dependent. Results of subsequent production experiments performed after infection of the modified cells with influenza B/Victoria/504/2000 virus showed that the cell-derived virus was antigenically similar to the chicken embryo-derived virus and its nucleotide sequence was identical. Cells expressing Siat7e produced hemagglutinin (expressed as hemagglutination units per 106 cells) with approximately 20-fold higher specific yields of hemagglutinin than those produced by parental MDCK cells.

4.3.3 MDCK Cell Culture for Influenza Virus Vaccines

Based on the suspension culture technique described above, it is possible to obtain different MDCK cells suitable for different production conditions by obtaining the virus from cells at different stages. If the virus is cultured in chicken embryos, it needs to be cultured in MDCK cells first to adapt to the cells. The cultured virus is added to the cells for co-culture, allowing the virus to enter the cells and pass on until the virus is stabilized. Finally, the cell suspension is collected, clarified filtered, purified by formaldehyde inactivation, and lysed to obtain the influenza virus vaccine.

5 TECHNICAL SUMMARY COMPARISON OF PRODUCTION METHODS (COMPARISON OF DISCOVERED VACCINE PRODUCTION METHODS)

In terms of production operation, the influenza

vaccine cultured in the chicken embryo has relatively less complexity of operation than the influenza vaccine cultured in MDCK cell culture, and it is found to be early, has a long period of use, and has a guaranteed safety, but in terms of product quality the cells cultured in MDCK cell culture are closer to the receptors of human cells. In addition, during viral proliferation, the instability of its RNA leads to changes in the aspartic acid residue sites of the expressed proteins, causing glycosylation shifts or leading to other mutations. This makes the produced vaccine weak or even ineffective. However, through the study of Li et al (Li et al 2021), it was found that the glycosylation sites and potential sites of viral HA proteins produced by MDCK cells were more than those of vaccines produced by chicken embryos, so MDCK cells were gradually selected for vaccine production.

6 CHALLENGES AND PROSPECTS

Limitations of utilizing chicken embryos for influenza vaccine production include (1) Lower production efficiency compared to cell culture making it difficult to meet the demand in case of an influenza pandemic. (2) Some findings suggest that the chicken embryo-adapted influenza virus strains used for vaccine production have three amino acid mutations in the antigenic sites B [H156Q, G186V] and D [S219Y] of the strains themselves compared to the prototype strains recommended by the World Health Organization and that these mutations may result in significant changes in the antigenicity, immunogenicity, and efficacy of the vaccine. (Skowronski et al, 2014) These challenges may allow further study of glycosylation of viral surface proteins. A limitation of influenza vaccine production with MDCK cells is that its safety needs to be improved. MDCK cells have been reported to have a potential risk of tumorigenesis in immunodeficient mice (Jin et al., 2024). The tumor genesis and influencing factors can be further investigated.

7 CONCLUSION

The purpose of this study is to summarize and introduce the production methods of influenza vaccine and to identify the problems of the existing production methods. Through this study, it was found that the process of human vaccine production is a race against the mutation of pandemic viruses, so in the future, more in-depth research can be conducted on the cultivation of influenza vaccines by other mammalian cells similar to MDCK cells, as well as due to the extensive use of gene technology, or can be genetically edited on existing MDCK cells, so that its receptor can be more compatible with that of human cells or can be combined with influenza viruses more smoothly, which can prepare for the emergence of more potent influenza viruses in the future. It is also hoped that in the future, it will be possible to make mammalian cells more resistant to influenza viruses so that they can be prepared for the emergence of more potent influenza viruses. It is also hoped that in the future, the vaccines produced by mammalian cells will be safer, more stable, and simpler, and can be customized for the mass production of different types of influenza. The ultimate goal is to be able to combine big data and artificial intelligence technology to predict the mutation of the virus so that the efficiency of the vaccine produced can be increased or even reach 100% effectiveness.

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