Rabies Virus Glycoprotein-Based DNA Vaccines: Current Status and Future Directions

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Abstract: Rabies is a very virulent viral infection, and rabies DNA vaccines are a novel approach to its prevention. These vaccines are prepared by placing the gene that codes for a key rabies virus antigen in a recombinant DNA plasmid under the control of eukaryotic ex-pression elements. When given to the body, they cause humoral and cell-mediated immune reactions with the following benefits: broad-spectrum immunity, long-term im-munity, fewer side - effects, easy production, and absence of requirements for cryopreservation. Nonetheless, DNA vaccines are limited by relatively weak immunogenicity relative to protein-based vaccines, uncertain long-term protection, and restricted mass production and regulatory approval. Ongoing research is aimed at enhancing immunogenicity, optimizing dosing regimens, simplifying production processes, and establishing appropriate regulatory infrastructures. These efforts will maximize the application of rabies DNA vaccines in rabies control globally.

1 INTRODUCTION

Rabies remains a critical public health threat, with nearly 100% mortality once clinical symptoms appear. The rabies virus is a neurotropic pathogen that primarily invades and replicates within the nervous system. Transmission occurs when infected animals bite, scratch, or lick broken skin or mucous membranes of humans (WHO, 2019). Upon entry, the virus's glycoprotein spikes bind specifically to receptors on host neuronal cells, facilitating viral entry. Inside the cell, the nucleoprotein orchestrates transcription and replication of the viral genome, leading to rapid viral proliferation. As viral replication disrupts neuronal function, impaired nerve signal transmission manifests clinically as hydrophobia, dysphagia, and respiratory difficulties. Progressive neuronal damage ultimately results in respiratory and circulatory failure and death. Rabies remains widely endemic across multiple regions, imposing significant health, economic, and social burdens globally (Whitehouse et al., 2023).

Rabies vaccines include human diploid cell vaccines, Vero cell vaccines, and primary cell vaccines, such as those derived from hamster kidneys and chicken embryos. While these vaccines have been instrumental in controlling the disease, they mainly stimulate humoral immunity and offer limited activation of cellular immune responses. The reliance on humoral immunity alone may not provide sufficient protection against viral infection, particularly in the context of emerging viral variants. This demonstrated the inefficacy of traditional rabies vaccines. DNA vaccines, on the other hand, showcase a stable alternative with the potential to stimulate both humoral and cellular immune responses. Furthermore, DNA vaccines are less expensive, more stable, easy to prepare, do not need cryogenic preservation, and are easy to popularize in developing countries (Fisher et al., 2018). This review aims to explore the key technologies and challenges in the development of rabies virus DNA vaccines and assess their potential in global vaccination. The comprehensive analysis of the existing literature is aimed to provide theoretical support for the improvement and popularization of the rabies virus vaccine in the future.

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2 STRUCTURE AND IMMUNE EVASION STRATEGIES OF THE RABIES VIRUSS

2.1 Structure and Mechanism of Infection

The rabies virus is a bullet-shaped, single-stranded, negative-sense RNA virus of the Rhabdoviridae family. It has a structure built up of a nucleocapsid core, with a surrounding lipid bilayer envelope. The surface glycoprotein is vital in the viral entry into the host cell. There are glycoprotein spikes that can specifically bind to receptors on the surface of host nerve cells, such as the nicotinic acetylcholine receptor. This binding event induces conformational changes of the glycoprotein involved in the fusion of the virus with the cell membrane and the entry of the virus into the cell by endocytosis. Inside the cell, the nucleoprotein releases the viral genome that starts viral transcription and replication. The virus hijacks the host's transcriptional machinery to produce viral proteins, ultimately leading to the assembly of new virions and their spread through neural pathways. This efficient invasion mechanism contributes to the high lethality of rabies infections (Sugiyama et al., 2025).

2.2 Immune Evasion Strategies

The rabies virus has developed sophisticated strategies to escape the host immune system. One primary mechanism involves its ability to persist within nerve cells, where immune surveillance is inherently limited. Unlike other tissues, the nervous system has restricted access to circulating immune cells, allowing the virus to replicate and spread largely undetected. Apart from this, the antigenic structure is continuously modulated by the virus. Over a period, the glycoprotein mutates, which causes changes in the epitopes recognized by the immune cells of the host, such as antibodies and T lymphocytes. Therefore, the immune system is not able to clearly recognize the virus, which allows the virus to carry on with its infection cycle and cause disease. This antigenic variation, combined with the virus's neurotropic nature, made the rabies able to start the infection while evading effective immune responses (Guo et al., 2019).

3 DNA VACCINE TECHNOLOGY FOR RABIES CONTROL

3.1 Principles of DNA Vaccines

DNA vaccines are extremely efficient in rabies prevention, offering advantages over traditional vaccines. DNA vaccines for rabies are constructed by placing the gene encoding a key antigen (which is often the glycoprotein of the rabies virus) under the control of eukaryotic expression elements that form a recombinant DNA plasmid. When this plasmid is introduced into the animal body, it enters host cells. Inside the host cell, the plasmid utilizes the cell's endogenous transcription and translation machinery. The eukaryotic promoter in the plasmid initiates the transcription of the inserted antigen-encoding gene into messenger RNA (mRNA). This mRNA is then translated into the antigen protein by ribosomes in the cytoplasm. The newly synthesized antigen protein is then presented on the cell surface or released into the extracellular environment. This process of the antigen activates the host's immune system, which then leads to the production of both humoral and cellular immune responses. B cells recognize the antigen and differentiate into plasma cells, which secrete antibodies. T cells, including cytotoxic T lymphocytes (CTLs), are also activated, which can directly kill virus-infected cells. This dual activation of humoral and cellular immunity provides broad and long-lasting protection against rabies virus (Mlingo et al., 2025).

3.2 Advantages of DNA Vaccines

Firstly, the DNA vaccine will have a benefit in terms of immunogenicity compared with traditional rabies vaccines. While traditional vaccines would mostly exert humoral immunity, DNA vaccines are capable of additionally activating cellular immune responses. Based on such broad immune activation, DNA vaccines provide greater protection against any viral infection, especially in the presence of their variants. Secondly, in terms of efficacy, DNA vaccines should give long-lasting protection, mainly due to the possibility of prolonged antigen production within the host cell. Traditional vaccines may have to be given repeatedly, for example, by multiple boosters, to maintain some immunity. In addition, DNA vaccine side effects are generally expected to be fewer than those inherited from traditional vaccines. They do not contain live or attenuated viruses, consequently

reducing the likelihood for the vaccine to cause some form of the disease itself or severe allergic reactions. Moreover, the DNA vaccines are relatively easy to manufacture. What is required is mainly plasmid amplification in bacteria, which is much more convenient and less costly compared to production processes of traditional vaccines, like growing viruses in cell cultures (Porter et al., 2017). Finally, in contrast to other vaccines, one does not have to worry about cryogenic preservation for DNA vaccines, which makes them warranted for use in developing countries, owing to the limited cold-chain structure.

4 OPTIMIZATION STRATEGIES FOR RABIES VIRUS DNA VACCINES

4.1 Enhancing Glycoprotein Immunogenicity

The glycoprotein is one of the most immunogenic antigens of the DNA vaccine for rabies and is thus the vaccine target since it is the main protein that the immune system recognizes in natural infection (Chen et al., 2025). Various optimization approaches have been employed to increase the immunogenicity of the rabies virus glycoprotein. Codon optimization has been employed to enhance translation efficiency in host cells, while fusion with immune-stimulating cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), has been investigated to enhance antigen presentation. Structural modifications, such as stabilizing the glycoprotein in its native trimeric conformation, have also been explored to improve its recognition by the immune system and increase the production of neutralizing antibodies (Ng et al., 2022).

4.2 Plasmid Design and Promoter Selection

Selecting the right plasmid plays an important role in the functionality of the rabies virus DNA vaccines. Different plasmids have different properties and making a well-considered choice can significantly affect the success of the vaccine. Promoters are yet another game-changer in plasmid-based vaccines. While strong promoters such as the cytomegalovirus promoter are effective for high-level expression of the antigen-encoding gene, there are specific contexts in which tissue-specific promoters may be more appropriate since they target expression of the antigen in specific types of cells to enhance the immune response. Some additional enhancers can also be transfected to help regulate gene expression through the plasmid. These elements work together to improve the production of the antigen protein within host cells that enhances the immune effect of the vaccine (Disis et al., 2023).

4.3 Delivery Systems for DNA Vaccines

Efficient delivery of DNA vaccines into host cells is critical for achieving robust immune responses. There are a number of delivery methods that can be utilized for DNA vaccines. Viral vectors, including adenoviruses, and lentiviruses, can be used to deliver the DNA plasmid. These vectors can successfully penetrate the host cell and release the plasmid inside. However, they may activate existing immune responses against the vector itself. Other methods of delivery involve the use of liposomes to encapsulate the DNA plasmid to protect it from degradation and assist with its entry into cells. Relatively safe, they can also be altered to target specific cell types. Electrically, this method provides a field in which temporary pores are produced by electric pulses transmitted between electrodes into the membranes of the cells; through the pores, DNA plasmids can get into the cells. All the different delivery approaches each have their inherent strengths and weaknesses, leading researchers to sporadically amuse themselves with techniques to optimize them with the hope of further rousing rabies virus DNA vaccine effectiveness.

5 CHALLENGES AND FUTURE PATHWAYS

5.1 Mitigation of Immunogenicity Constraints

Although promising possibilities of DNA vaccines, these mainly produce more timid immune responses as opposed to protein-based ones. The primary reason lies in intricate processes of antigen expression and presentation after the DNA vaccine has achieved entry into cells and are guided by different variables. In addressing the weakness, scientists turn towards several avenues. On the one hand, more optimization of the antigen gene design is underway. For example, modification of the amino acid makeup of the antigen

can enhance the immune system recognition. On the other hand, studies on new adjuvants are ongoing. The adjuvants have the potential to enhance the ability of the immune cells to absorb and process antigens and to make the immune response stronger.

5.2 Fulfilling Long: Term Protection and Booster Needs

While DNA vaccines can extend antigen expression, the long - term duration of immunity is an issue. The research today aims to identify the best dosing regimen and if booster doses should be administered periodically to ensure protective immunity. Researchers perform animal and clinical studies, varying dosing frequencies, dosage, and route of administration, to monitor variations in the immune response. Meanwhile, they are studying vector systems that can stably and continuously express antigens, so the immune system is always stimulated and maintains a high level of immune protection. Meanwhile, research on the generation and maintenance mechanism of memory immune cells is carried out to provide a theoretical basis for making reasonable booster immunization schedules.

5.3 Large-Scale Production and Regulatory Considerations

In order to apply them on a large scale, DNA vaccines must be produced inexpensively in large lots and undergo stringent regulatory evaluations to establish efficacy as well as safety. For now, the vast majority of DNA vaccine production relies on bacterial fermentation for plasmid amplification, which however still remains challenging such as cost-prohibitive and yield-fluctuating protocols. In the future, there is a necessity to optimize the production process, increase the yield and purity of plasmids, and reduce production costs. Existing regulatory frameworks may not fully accommodate DNA vaccines, given their status as a novel vaccine class. Therefore, there is a need to create a specialized regulatory system for DNA vaccines, balance safety and efficacy assessment, and simplify production and the approval process, which will be paramount for their global application.

6 CONCLUSION

Rabies is a deadly viral infection that dangerously threatens human and animal health. Rabies vaccines have played an important role in preventing and controlling rabies, but with certain drawbacks. DNA vaccines, a new form of rabies protection, possess the following unusual strengths. By positioning the gene responsible for coding an important antigen (such as glycoprotein) of the rabies virus onto a recombinant DNA plasmid governed by eukaryotic regulatory elements, they can activate both the cellular and humoral host immune responses once inside the organism and thereby bestow broad-spectrum and durable immunity. Relative to traditional vaccines, DNA vaccines possess significant advantages in immunogenicity, efficacy, side - effects, ease of production, and storage needs. However, currently, DNA vaccines also possess many issues in practical applications, such as comparatively weak immunogenicity, requiring extending the duration of immunity, and encountering difficulties in mass production and approval by regulatory agencies. Future research needs to be more profound, like maximizing vaccine design, increasing production processes, and streamlining the regulatory system, to maximize the full potential of DNA vaccines in prevention and control of global rabies and safeguard the life and health of human beings and animals.

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