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# Research Advancement in RV Novel Vaccine

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## ABSTRACT

In this study, we reviewed the international research progress on the novel vaccines of rabies. Rabies is a lethal infectious disease, causing nearly 55,000 deaths worldwide each year. To date, pre-exposure vaccination is the most effective method to prevent rabies. As most of rabies cases occurred in developing countries, it is necessary develop vaccines that are inexpensive; furthermore, eradicating of rabies in wildlife calls for one single injection of vaccine. In the present study, vaccines employed for vaccination, including inactivated vaccines, subunit vaccine, DNA vaccine and newly attenuated vaccine are reviewed. In addition, viewpoints about development trends of vaccines are also discussed here. Since, rabies virus has wide range of reservoirs and vaccines now used is targeted to specific animal; in addition, the potential of reversion to pathogenic rabies virus makes it urgent need to develop new types of vaccines applying to different kinds of animal species. Also, new vaccines must be able to confer protective immunity with one single immunization of vaccines, because of difficulty in finding rabies virus reservoir for booster vaccinations.

**Key words:** Rabies virus, vaccine, advancement, research

## INTRODUCTION

Rabies is a severe encephalomyelitis viral disease throughout the world which affects warm-blooded animals and humans. Rabies is thought to be one of the oldest diseases of mankind. The human implications of the disease are found in early Egyptian hieroglyphics and the writings of Asclepiadae, Democritus, Aristotle and others (Steele and Femandez, 1991). The morality of rabies is almost 100%. It is reported nearly 55,000 human die from rabies all over the world each year (Knobel *et al*., 2005). Rabies virus not only results in economic loss but also represents a serious public health threat in developing countries. Therefore, it is important to prevent, control and eradicate rabies.

Vaccine plays an instrumental role in prevention and control of infectious disease. More than 100 years ago, Louis Pasteur developed a crude nervous tissue vaccine which saved the life Joseph Meister in 1885 (Hoeniq, 1986). Since, then, various types of inactivated virus vaccines derived from cultured cells, such as the human diploid cell vaccine (Wiktor *et al*., 1969). The purified chick embryo cell vaccine (Barth *et al*., 1984) and human diploid cell culture rabies vaccine (Dietzschold and Hooper, 1998).

At present, the vaccine widely employed for rabies is inactivated vaccine. However, the production process runs a risk of revealing live rabies virus to the environment which should be considered seriously in areas free of rabies. The residual pathogenicity of conventional modified live vaccines derived from the SAD strain was confirmed (Artois *et al*., 1992). Besides, short shelf life, the need of adequate cold chain have limited the use of inactivated vaccine. New types of vaccines are needed to solve the problem. In the present study, according to the mechanism, form and source of vaccine, the latest research progress of novel vaccine were reviewed; also, some viewpoints for the development of rabies vaccines fall within the scope of the study.

## SUBUNIT VACCINE

Subunit vaccine is a vaccine that contains isolated antigenic proteins from virus but lacks viral nucleic acid. These vaccines expose the body to antigens, so it can recognize them without exposing the body to the risk of viral replication and subsequent infection. The primary advantage of using a subunit vaccine is that it is very safe, even in people with comprised immune system, because it does not contain the genetic material of the virus, only the proteins on the exterior of the virus.

It is indicated that Glycoprotein (G), the surface protein of rabies virus, is the primary antigen responsible for inducing the immune response of the host. In earlier years, G produced in *Escherichia coli* (*E. coli*) could not offer protective immunity because lack of glycosylating (Yelverton *et al*., 1983; Lathe *et al*., 1994). Interestingly, the G protein produced in *Saccharomyces cerevisiae* offered protection only via intramuscular but not intracerebral virus challenge although, G has been glycosylated (Klepfer *et al*., 1993). It was thought that G has not been correctly refolded. Based on the idea of correct refolding, Prehaud *et al*. (1989) cloned the G gene of rabies virus, transferred into *Baculovirus* vectors and the gene was highly expressed in *Spodoptera frugiperda* cell line. The G conferred protection to mice and was associated with the induction of high titer of neutralizing antibodies. Avian adenovirus expressing G of rabies virus has good perspective for domestic and wild animal vaccination, not only due to high protection level but also because the production adenoviral vaccine in chicken embryos is of high technology and inexpensive (Shmarov *et al*., 2006). Vaccines based on recombinant pox viruses hold greatest promise of safety and efficiency. Although, these vaccines expressing G induced protective immunity in foxes, they were less efficient in other animals and safety concern has been raised for some of these vaccines (Li *et al*., 2006). Mice vaccinated with recombinant vaccinia virus expressing the G and nucleoprotein (N) developed strong antibody responses and survived challenge infection, 59% protection was observed in mice vaccinated with virus expressing phosphoprotein (P) and none could survive vaccinated with virus expressing matrix protein (Takita-Sonoda *et al*., 1993).

Because canine adenovirus 2 (CAV2) is licensed for use as a live vaccine for dogs and has an excellent efficacy and safety record, Li *et al*. (2006) used this virus as an expression vector for G of rabies virus. A single immunization of mice with CAV-RVG induced protective immunity in a dose-dependent manner.

Since 1990, plants have been considered as a promising alternative to produce safe and effective pharmaceuticals at low cost. In case of rabies, Mc Garvey *et al*. (1995) reported stable expression of G in transgenic tomato without immunoprotective ability Mc Garvey *et al*. (1995). Later, by replacing the signal peptide with PR-S of *Nicotiana tabacum* and including the endoplasmic reticulum retention signal, G was expressed in tobacco. The tobacco-derived G induced complete protective immunity in mice against intracerebral lethal challenge (Ashraf *et al*., 2005).

The N of rabies virus is highly antigenically conserved and is a better candidate for the development of vaccine (Mannen *et al*., 1991). It was demonstrated N induced protective immunity against a peripheral challenge with rabies virus (Lodmell *et al*., 1991). Also, N expressed in insects by *Baculovirus* vectors induced antibodies in mice (Prehaud *et al*., 1990). Fu *et al*. (1991) demonstrated immunization of N expressed in insect cell developed a strong anti-ribonucleoprotein antibody and could protect mice against lethal challenge of rabies virus.

Post-exposure vaccination with vaccinia virus recombinant expressing the G conferred protection when given immediately following infection, in contrast, vaccinia virus expressing N failed to confer protection even with high doses (Fujiii *et al*., 1994).

In order to be effective for rabies virus, amino acid sequence comprising the antigenic determinant for Virus Neutralizing Antibody (VNA) binding must be folded properly. Also, vectors should be considered seriously, recombinant vaccinia virus, adenovirus and *Baculovirus* failed to develop rabies vaccine because of safety concerns (Rupprecht *et al*., 1983; Prevec *et al*., 1990; Prehaud *et al*., 1989).

## LIVE VECTOR VACCINE

A live vector vaccine is a vaccine that uses a chemically weakened virus to transport pieces of the virus in order to stimulate an immune response. Live vector vaccines have advantage over other vaccines in that they are innocuous because infective rabies virus particles are not handled and they elicit both cellular and humoral immune response (Blanton *et al*., 2006).

Adenovirus is one of the most widely virus to express heterologous genes. The structure and function of adenovirus genome has been studies thoroughly and in 1990, human adenovirus type 5 recombinant virus expressing the rabies G was constructed. The recombinant virus was highly effective in eliciting high level of VNA in dogs and mice (Prevec *et al*., 1990). Later, replication-defective recombinant human strain-5 adenovirus expressing G has been constructed. Immunization of neonatal mice with the recombinant virus induced long-lasting immunity, indicating the possible use of recombinant virus for immunization shortly after birth (Wang *et al*., 1997).

Live modified canine adenovirus type 2 (CAV2) which has been widely used for vaccination of dogs against CAV1 has an excellent safety record, is an ideal vaccine vector for immunization of carnivores against rabies (Fischer *et al*., 2002). CAV2 expressing G of rabies virus can be used as a live vector against rabies in swine and potent humoral immune response is elicited (Liu *et al*., 2008). A crucial criterion for the efficiency of the CAV2-RVG is low natural prevalence of CAV2 in the population to be vaccinated. Li *et al*. (2006) constructed CAV2 expressing the G of rabies virus. The recombinant CAV 2-RV G produces virus titers similar to those produced by wild-type CAV 2, a single immunization of mice with CAV 2-RV G induced protective immunity, regardless of the route of immunization and the amount of virus. Also based on CAV, Hu *et al*. (2007) demonstrated a single, intramuscular dose of CAV-2-E3$_{\Delta}$-RGP (canine adenovirus-rabies vaccine) stimulated a long-lasting protective immune response in cats, indicating CAV-2-E3$_{\Delta}$-RGP could be considered as a potential rabies vaccine candidate for cats.

A recombinant vaccinia virus expressing the immunizing G of rabies virus has been developed to improve safety and stability of the vaccine used. Because of its efficacy, innocuity and heat-stability, the vaccine seems to offer an excellent alternative to the attenuated strains of rabies virus currently used in the field (Brochier *et al*., 1995).

Several studies have explored the feasibility of using plant viruses as expression vectors for vaccine antigens. It is shown mice immunized with recombinant virus recovered from infected tobacco have been protected against a lethal challenge infection with rabies virus. Human volunteers fed raw spinach leaves containing experimental plant virus-based vaccine developed a rabies virus specific immune response (Yusibov *et al*., 2002). The modified vaccinia virus Ankara (MVA) expressing the G of rabies virus was constructed. The recombinant vaccine proved immunogenic upon peripheral administration in mice at the cost of efficiency and the lack of oral innocuity of the recombinant MVA rabies G would, thus rule this candidate unsuitable for replacement of other rabies vaccines (Weyer *et al*., 2007).

Live recombinant vaccine holds the promise of safety and efficacy for rabies. In particular combination with other vaccine, such as DNA vaccines or subunit vaccine, showing to be an effective way to enhance antibody titers.

**DNA VACCINE**

Since, its early applications in the 1950's, DNA-based immunization has become a novel approach to vaccine development. DNA vaccine is one of novel vaccine based on DNA. It is a technique for protecting an organism against disease by injecting with genetically engineered DNA to produce an immunological response. Plasmid containing the optimized gene is injected into the host, then the plasmid is taken up and plasmid-encoded genes are expressed to generate the antigens. The antigen can stimulate both humoral and cellular immune response (Danko *et al*., 2011). DNA vaccine has applied to a number of viral, bacterial and parasitic models of disease, as well as several tumor models. DNA vaccine is attractive not only because the DNA does not replicate but also because the DNA is stable and can be made inexpensively in large quantities at high levels of purity (Pardoll and Beckerleg, 1995). There are researches showing plasmids encoding G of rabies have been reported to induce immune responses in mice and non-human primates against rabies virus lethal challenge (Xiang and Ertl, 1994; Xiang *et al*., 1994; Bahloul *et al*., 1998; Lodmell *et al*., 1998; Biswas *et al*., 1999; Osorio *et al*., 1999; Perrin *et al*., 1999), in the post-exposure vaccination of mice (Lodmell and Ewalt, 2001; Tesoro-Cruz *et al*., 2008) even without anti-rabies virus maternal antibody. Mice immunized with DNA vaccine were protected as neonates (Wang *et al*., 1998).

Promoter is an important parameter which may affect the efficacy of DNA vaccine. Xiang *et al*. (1995) demonstrated the vectors with promoter SV40 and CMV showed striking difference in the ability to stable expression of the rabies virus G protein but the magnitude of immune response was similar.

Sites of injection are deeply related to the potency of vaccine. In non-human primates and cats vaccination at ear pinnae was the most effective site and intradermal (i.d.) route of vaccination was the most effective way of vaccination (Tesoro-Cruz *et al*., 2008; Lodmell *et al*., 2002). Similar results have been reported by Forq *et al*. (1998) and Lodmell *et al*. (2003).

Routes of vaccination are deeply involved in enhancing the potency of vaccines. Ray *et al*. (1997) demonstrated that both intradermal and intramuscular needle injection of plasmid encoding the G of the Challenge Virus Standard (CVS) rabies virus offered protective immunity against lethal rabies virus infection. However, it seems i.d. inoculation in cats is more effective than other routes of vaccination. This finding is consistent with previous reports (Raz *et al*., 1994, 1996; Sato *et al*., 1996). Later, it was found DNA vaccine intranasally applied was more effective as a post-exposure prophylaxis using only four doses and without the application of rabies immune globulin than the use of a traditional vaccine (Tesoro-Cruz *et al*., 2008).

Traditional vaccines call for more than one vaccination, to enhance the titer virus neutralizing antibody. However, a single DNA vaccination elicits life-long protection of mice against rabies virus (Lodmell and Ewalt, 2000). It was also demonstrated a single injection using jet injector of DNA vaccine induced higher levels of neutralizing antibody than multiple injections of the best commercially available cell culture-derived vaccine (Bahloul *et al.*, 2006).

Co-inoculation of vaccines with cytokines has been shown to enhance the immune response of host. Xiang and Ertl (1995) reported co-inoculation with a vector expressing mouse GM-CSF enhanced the B and T helper cell activity of rabies virus while co-inoculation with a vector expressing IFN-γ led to the decrease of the immune response. Pinto *et al.* (2003) proved some of chemokines augment both B and T helper cell response to the vector encoded glycoprotein. Later, the team indicated that single delivery of 2 μg of DNA coated on 2.6 μm gold beads induced high level of rabies VNA to confer protective immunity. Three hundred and fifteen days post-primary immunization, 100% of the mice survived intraplantar rabies virus challenge (Lodmell *et al.*, 1998).

Vectors of DNA vaccine including plasmids and virus. In 2008, a sindbis virus replicon-based DNA vaccine encoding the G of rabies virus was developed. The mice immunized with replicon-based rabies DNA vaccine induced humoral and cell mediated immune response better than conventional rabies DNA vaccine (Saxena *et al.*, 2008).

The field of DNA vaccine has advanced significantly in the last two decades. Various attempts to improve the potency of plasmid DNA vaccine, including the DNA construction, co-immunization with adjuvants, alternative routes of vaccination have been made. Based on the data obtained so far, it is believed DNA vaccine is a promising alternative to the conventional vaccines.

## NOVEL LIVE ATTENUATED RABIES VACCINE

Oral immunization of wildlife with live vaccines has proved to be the most effective method to control and eradicate rabies. However, concerns regarding the safety of live vaccines have been raised since 2000, when a person who had been vaccinated with VRG developed severe inflammatory reaction and axillary adenitis and later generalized erythroderma (Rupprecht *et al.*, 2001). Therefore, safety is the most important consideration in live vaccine. With the development of reverse genetics technology, it is possible to develop new attenuated live vaccine. Morimoto *et al.* (2001) used different modified rabies virus G genes to engineer rabies recombinant viruses. The recombinant viruses were nonpathogenic but differed greatly in ability to induce protective immunity. Based on Dietzschold and Schnell (2002) developed novel live rabies vaccine, the highest level of protection obtained was 90%.

As G is the principal antigen inducing the VNA of the host, recombinant virus with more than one G gene or deleting the has been firstly constructed. Recombinant Flury Low Egg Passage virus (LEP) carrying two identical G gene was constructed by Tao. The inactivated vaccine generated from rLEP induced significantly higher virus neutrahizing titers in mice than LEP derived vaccine, suggesting rLEP is candidate for inactivated virus vaccine manufacture (Tao *et al.*, 2011). RV including $Arg_{333} \rightarrow Glu_{333}$ mutation is nonpathogenic for mice and $Asn_{194} \rightarrow Ser_{194}$ mutation prevents reversion to pathogenic phenotype (Faber *et al.*, 2005; Dietzschold *et al.*, 2004). A recombinant RV containing two identical glycoprotein carrying both of two mutations could induce mice higher antibody titers against glycoprotein (Faber *et al.*, 2002). And RV expressing 3 copies of both mutations could not only induce protective immunity after a single immunization but also is nonpathogenic for very young mice. Lack of pathogenicity, as well as perfect immunogenicity and deliver immune effectors makes the recombinant virus promising candidate for both pre-exposure and post-exposure prophylaxis of rabies (Faber *et al.*, 2009).

Pulmanausahakul *et al.* (2001) constructed recombinant rabies virus expressing cytochrome c gene. The strong increase in immunogenicity, coupled with marked reduction in pathogenicity, proving the recombinant virus a candidate for a live rabies virus vaccine.

P has been thought to be related to the pathogenicity of rabies virus. Thus, Rabies virus deficient in the P gene may be developed to new type of vaccine. Shoji *et al.* (2004) found rabies virus deficient in P was apathogenic in adult and sucking mice. High levels of virus neutralizing antibody was induced to protect against lethal rabies infection, demonstrating the potential utility of gene-deficient virus as a novel hive attenuated rabies vaccine. However, a single dose of M gene-deficient RV induced a more rapid and efficient anti-RV response than P gene-deficient RV, although both virus are immunogenic and provide protection against pathogenic RV challenge for pre-exposure (Cenna *et al.*, 2009). Cenna *et al.* (2008) described a recombinant P-deleted virus that expresses two copies of the RV glycoprotein gene. Of note, $10^3$ FFU of the recombinant virus induced antibodies which were 100% protective in mice against pathogenic RV challenge.

It is evident that by $Arg_{333} \rightarrow Glu_{333}$ mutation, the novel live attenuated based on the reverse genetics engineering is very safe. With the improvement in the potency, it may be a useful vaccine candidate in the coming years.

## SYNTHETIC PEPTIDE VACCINE

Synthetic peptide vaccine is vaccine using synthetic short peptide corresponding to major epitopes of viral proteins to elicit a protective antibody response.

Dietzschold *et al.* (1990) found that a synthetic peptide containing the amino acid sequences of the 6-15C4 epitope and the dominant 31D T-cell epitope in tandem induced a significant protective response in C3H mice against a lethal rabies virus challenge, suggesting that it may be possible to produce a synthetic peptide vaccine against rabies. However, the synthetic peptide encompassing the G5 antigenic site did not show a T helper cell proliferative response and the antibody titers is low. The peptide containing T-cell epitope and B-cell epitope is capable of inducing robust T-cell response and provide help for a strong antibody response to B-cell epitope (Fluri *et al.*, 2006). Both B-cell and T-cell epitopes have been mapped throughout the N protein (Minamoto *et al.*, 1994; Goto *et al.*, 1995). Subsequently, fusion protein comprising one B-cell and one T-cell epitope has been reported by Da Cruz *et al.* (2001). The fusion protein elicited a higher antibody titer than that of rabies vaccine (Da Cruz *et al.*, 2001).

## RECOMMENDATIONS

Rabies virus is not a single entity but of a wide variety of variants which are associated with different host species. Rabies vaccines available have been greatly targeted to individual animal species because of this diversity. Therefore, it is necessary to develop new type of vaccines applying to different kinds of animal species. In addition, the poor vaccination coverage, in addition to the difficulty in finding rabies virus reservoir for booster vaccinations, calls for an inexpensive rabies vaccine eliciting long-term immunity with a single vaccination.

Vero cells are employed for rabies virus vaccine production. The data based on of the experiment, the Vero cell derived rabies vaccine production is encouraging (Jagannathan *et al.*, 2009). Also, Hassanzadeh *et al.* (2011) achieved higher Vero cell density for vaccine production and rabies virus proliferation was fairly well. Vaccine in future must be absolutely safe and able to confer long-lasting immunity after a single administration. The inactivated aluminum-adjuvanted rabies vaccines (IARV) which is currently being used to immune dogs to control rabies, failed to induce protective immunity by a single injection of the vaccines (Minke *et al.*, 2009).

Because of the ability to induce strong innate and adaptive immune responses capable of clearing the infection from the CNS after a single immunization, live-attenuated rabies virus vaccines could be particularly useful not only for global eradication of canine rabies but also for large-scale postexposure prophylaxis of human.

A live rabies virus vaccine should fulfill the following criteria: (1): it must not cause disease, regardless of the route of infection and immune status of the host and (2): it must confer sufficient protective immunity after oral immunization and the virus must produce high virus titers in tissue culture cell lines which is a prerequisite for economics production. Therefore, great efforts are needed to agree with these criteria.

New kinds of adjuvants which could be used to enhance the potency of the vaccine, needs to be developed. Also, immunization strategies could also be developed. It was reported that combination immunization with DNA vaccines and subunit vaccines could induce high titer antibody and cellular immunity. This is a shortcut to improve potency of vaccine, because the development of new kind of vaccines is rather slow and combination of different vaccines available may be a choice.

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