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Research Article

EXPRESSION OF VIRULENT RABIES VIRUS GLYCOPROTEIN GENE CLONED IN pALPHA VECTOR

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ABSTRACT

Rabies virus virulent strain glycoprotein gene cloned in pAlpha vector was found to express to high level in BHK21 cell culture when examined by indirect fluorescent antibody and indirect immunoperoxidase tests. The mock transfected vector alone and healthy control cell cultures did not show any expression.

Key Words: Rabies virus, glycoprotein, gene expression

INTRODUCTION

Rabies is caused by a virus, which is negative stranded RNA virus of *Lyssavirus* genus in the family of *Rhabdoviridae*. Although conventional vaccines against rabies are available, these are not ideal for mass vaccination in developing countries like India. Out of five proteins encoded by rabies genome, glycoprotein is the only protein capable of inducing and reacting to virus neutralizing antibodies (VNA) and for conferring protective immunity against lethal challenge with rabies virus. This has laid down the possibility of developing a subunit vaccine

against rabies. DNA vaccines against rabies have been reported and found to be successful as pre-exposure and post exposure vaccines in mice (Perrin *et al.*, 2000; Lodmell and Gwalt, 2001; Rai *et al.* 2002, 2006; Lodmell *et al.*, 2002), dogs (Forg *et al.*, 1998), cats (Akbari *et al.*, 1999) and non-human primates (Krieg, 2000).

Ever since the discovery of Wolff *et al* (1990) that naked DNA injection into the muscle of mice led to expression of the encoded protein, many groups have successfully applied this technique to develop DNA vaccine against infectious pathogens in different animal models

(Ulmer *et al.*, 1993; Lagging *et al.*, 1995; Lowrie *et al.*, 1994; Major *et al.*, 1995; Michel *et al.*, 1995; Gupta *et al.*, 2001). With the help of recombinant DNA technology, plasmid expression vector carrying gene of interest is delivered and expressed in animal muscle. The DNA as a vaccine assumes great significance since it can offer protection against dreadful infectious diseases in developing countries besides being temperature stable and affordable for the poor when compared to recombinant/ cell culture vaccines. *In vivo* synthesis of antigen structurally identical to those produced during active viral infection and induction of strong cell mediated immune responses renders DNA vaccine advantageous over conventional vaccines. In addition, DNA vaccines are non-infectious, economical to produce in large amount and easy to purify using simple and inexpensive techniques. Further, DNA vaccines do not require cold chain, which occupies about 80% of the cost of vaccination in developing countries. Plasmids encoding multiple antigens of same pathogen or different pathogens can be constructed. DNA vaccines can be given at a small age without the risk of neutralization by maternal antibodies. Moreover, booster doses can be given without risk of vector immunity. Out of five proteins encoded by rabies virus genome, the glycoprotein (G protein) is the only viral protein that induces virus neutralizing antibodies (Cox *et al.*, 1977). G protein of rabies virus CVS strain was earlier cloned and shown to protect mice (Rai *et al.*, 2002).

It is logically acceptable that a vaccine should be developed which could

function in low dose with simultaneous induction and maintenance of strong immunity. The replicase based DNA vaccines fulfill the stringent criteria for all DNA vaccine in general (Hariharan *et al.*, 1998) and vaccines for breaking immunological tolerance (Leitner *et al.*, 2002). Transfection of host cells with replicase based genetic vaccine can trigger a series of danger signals (Matzinger, 1998) leading to apoptosis of infected cells. These apoptotic cells may be picked up by dendritic cells for presentation to the immune system. Transfection with self-replicating gene vaccine may also cause the production of heat shock proteins in transfected cells. The activity of viral replicase provides a powerful adjuvant effect because of the requisite production of double stranded RNA intermediates (Hariharan *et al.*, 1998).

Considering the significance of DNA vaccines based on replicase vector, the present study has been designed with the aim of construction of recombinant plasmid which could be utilized as DNA vaccine.

MATERIALS AND METHODS

Recombinant plasmid

The pAlpha-rvvg recombinant plasmid was constructed and available in the Biotechnology lab of IBIT Bareilly (Kanojia and Rai, 2016).

Cell Culture

Baby hamster kidney (BHK-21) cell culture maintained in Biotechnology lab was used in the study for *in vitro* expression analysis of recombinant plasmids and was maintained in DMEM (Gibco, NY) supplemented with 50 µg/ml gentamicin

(Amresco, USA)) and 10% new born calf serum (Gibco, NY).

Transfection of cell culture with plasmid DNA

Calcium phosphate mediated transfection of cell culture- BHK21 with plasmid DNA in microtitre plate was used (Rai *et al.*, 2014). Harvested exponentially growing MDCK cells by trypsinization and prepared cell suspension using growth medium. Prepared the calcium phosphate DNA co-precipitate as follows: Combined 100 μ l 2.5 M CaCl_2 with 20 μ l plasmid DNA in a sterile microfuge tube. Added 80 μ l distilled water. Kept at room temperature for 1 min and immediately transferred the calcium phosphate DNA suspension using 20 μ l suspension for each well of the microtitre plate. Added 100 μ l cell culture suspensions in each well. Rocked the plate gently to mix the medium which becomes yellow orange and turbid (carry out this step as quickly as possible because the efficiency of transfection declines rapidly once the DNA precipitate is formed). Kept the mock transfected (vector alone) and control healthy wells without transfection as well. Incubated at 37°C in a humidified incubator with an atmosphere of 5% CO_2 for 72 h.

Preparation of hyperimmune serum against rabies G

Primary antibody against the rabies glycoprotein G gene was raised in mouse by hyper immunization of six mice with pAlpha-rvvg plasmid DNA, 50 μ g per mouse injected intramuscularly in quadriceps muscle of thigh of each mouse and repeated every week for four weeks consecutively. Mice were bled through inner

canthus of eyes with a capillary and serum was prepared.

Indirect fluorescent antibody test (IFAT)

After 48 h transfection, media from the wells was poured off and the cells were washed twice with 1X PBS and fixed with 4% paraformaldehyde. Mouse primary anti-rabies glycoprotein hyperimmune sera (1:50 dilution) was added in triplicate wells of rplasmid, vector alone and healthy groups. It was incubated at 37°C for 2 h after which the wells were washed with 1X PBS and incubated with 1:40 dilution of FITC conjugated Secondary antibody goat anti-mouse for 2 h. After incubation the wells were washed with 1X PBS, mounted with 50% glycerol in PBS and examined under fluorescent microscope (Nikon) and photographed.

Immunoperoxidase test (IPT)

The transfected cells were washed with PBS and fixed with chilled acetone. The PBS washed cells were treated with 2% H_2O_2 for 10 min and again washed with PBS. The cells were first incubated with 1:50 dilution of anti-mouse rabies glycoprotein hyperimmune serum raised in mouse for 2 h at 37°C, washed three times with PBS and then incubated with rabbit anti-mouse HRPO conjugate (1:100 diluted) at 37°C for 2 h. The cells were again washed thrice with PBS and incubated with 3, 3'-diaminobenzidine (DAB 1mg/ml in PBS with 1 μ l/ml H_2O_2) for 5 min at room temperature to develop colour. Once the colour developed, the cells were washed with PBS, dried in air and were observed under microscope.

RESULTS AND DISCUSSION

The immunofluorescence analysis of pAlpha-rvvg transfected BHK-21 cells using anti-rabies glycoprotein hyperimmune serum and FITC-labeled secondary antibody revealed granular fluorescence, which indicated the expression of rabies virus glycoprotein (Fig.1). There was no fluorescence when mock transfected (vector alone) and healthy control cells were probed using same primary antibody and FITC labeled secondary antibody (Fig.2, 3). The results of immunoperoxidase test revealed granular brown colour deposits in pAlpha-rvvg plasmid transfected BHK-21 cells (Fig.4) when observed under microscope, whereas no such deposits were observed in mock transfected and vector control BHK-21 cells (Fig.5, 6).

In vitro expression of the recombinant plasmid was confirmed by indirect fluorescent antibody test, indirect and immunoperoxidase test. When the transfected cells were analysed with indirect fluorescent antibody test granular fluorescence was observed which indicated the presence of the glycoprotein in the endoplasmic reticulum, golgi bodies and the cell surface and that it was undergoing post-translation modification. Likewise, the immunoperoxidase test also revealed granular deposits in the cytoplasm of transfected cells, which confirmed the expression of the glycoprotein.

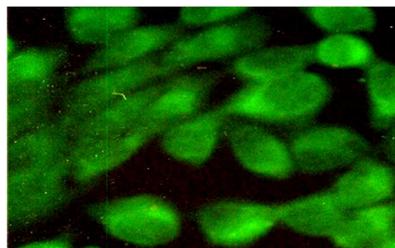


Fig.1. Indirect immunofluorescence of BHK21 cells transfected with pAlpha-rvvg plasmid DNA.



Fig.2. Indirect immunofluorescence of mock transfected BHK21 cells.

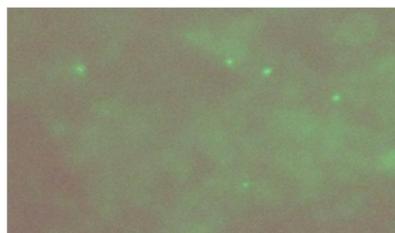


Fig. 3. Indirect immunofluorescence of control BHK21 cells.

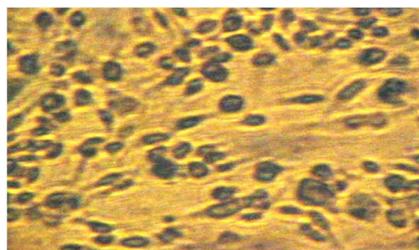


Fig. 4. Indirect immunoperoxidase test of BHK21 cells transfected with pAlpha-rvvg plasmid DNA.



Fig. 5. Indirect immunoperoxidase test of mock transfected BHK21 cells.



Fig. 6. Indirect immunoperoxidase test of control BHK21 cells

REFERENCES

- Akbari O, Panjwani N, Garcia S, Tascon R, Lowri D and Stockinger B. (1999). DNA vaccination: Transfection and activation of dendritic cells as key events for immunity. *J. Exp. Med.* 189: 169-178.
- Cox J H, Dietzschold B and Schneider L G. (1977). Rabies virus glycoprotein II. Biological and serological characterization. *Infect. Immun.* 16: 754- 759.
- Forg P von, Hoegen P, Dalemans W and Schirmacher V. (1998). Superiority of the ear pinna over muscle tissue as site for DNA vaccination. *Gene Therapy* 5: 787-797.
- Gupta P K, Saini M., Gupta LK, Rao V D P, Bandyopadhyay S K, Butchaiah G, Garg GK and Garg S K. (2001). Induction of immune responses in cattle with a DNA vaccine encoding glycoprotein C of bovine herpesvirus-1. *Vet. Microbiol.* 78: 293-305.
- Hariharan M J, Driver D A and Townsend K. (1998). DNA immunization against Herpes simplex virus: enhanced efficacy using a Sindbis virus based vector. *J. Virol.* 72: 950-958.
- Kanojia A and Rai A. (2016). Amplification and cloning of rabies virus virulent glycoprotein gene in pAlpha vector. *Biotechnology International.* 9 (1): 1-10.
- Krieg A M.(2000). Immune effects and mechanisms of action of CpG motifs. *Vaccine* 19: 618-622.
- Lagging L M, Meyer K, Hoft D, Houghton M, Belshe R B and Ray R. (1995). Immune responses to plasmid DNA encoding the hepatitis C virus core protein. *J. Virol.* 69: 5859-5863.
- Leitner W W, Leroy N, Hwang Michael, J., deVeer, Aimin Zhou, Robert H., Silverman, Bryan R.G., Williams, Thomas W., Dubensky, Han Ying, and Nicholas P.R. (2002). *Nature Medicine* 9: 33-39.
- Lodmell, D.L. and Gwalt, L.C. (2001). Post exposure DNA vaccination protects mice against rabies virus. *Vaccine* 19: 2468-2473.
- Lodmell D L, Parnell M J, Bailey J R, Ewalt L C and Hanlon C A. (2002). Rabies DNA vaccination of non-human primates: post exposure studies using gene gun methodology that accelerates induction of neutralizing antibodies and enhances neutralizing antibody titers. *Vaccine* 20: 2221-2228.
- Lowrie D B, Tascon R E, Colston M J and Silva, C L. (1994). Towards a DNA vaccine against tuberculosis. *Vaccine* 12: 1537-1540.
- Major M E L, Vitvitski M A, Mink M, Schleef R G, Whalen C T and Inchauspe G. (1995). DNA-based immunization with chimeric vectors

- for the induction of immune responses against the hepatitis C virus nucleocapsid. *J. Virol.* 69: 579865805.
- Matzinger P. (1998). An innate sense of danger. *Semin. Immunol.* 10: 399-415.
- Michel M L, Davis H L, Schleef M, Mancini M, Tiollais P and Whalen R G. (1995). DNA-mediated immunization to the hepatitis B surface antigen in mice: aspects of the humoral response mimic hepatitis B viral infection in humans. *Proc. Natl. Acad. Sci. USA.* 92: 530765311.
- Perrin P, Jacob Y, Aguilar-Setien A, Loza-Rubio, E., Jallet, C., Desmezieres, E., Aubert, M., Cliquet, F. and Tordo, N. (1999). Immunization of dogs with a DNA vaccine induces protection against rabies virus. *Vaccine* 18: 4796486.
- Perrin P, Jacob Y and Tordo N. (2000). DNA- based immunization against lyssaviruses. *Intervirology* 43: 302-311.
- Rai A, Gupta P K and Rai N. (2002). Cloning of rabies virus glycoprotein gene in a mammalian expression vector and immunogenicity of the recombinant plasmid DNA. *Indian J. Comp. Microbiol. Immunol. Inf. Dis.* 23 : 123-126.
- Rai N, kaushik P and Rai A. (2006). Expression of cloned rabies glycoprotein gene in mammalian cells. *Ind. J. Virol.* 17: 18-22.
- Rai A, Tiwari A K, Gupta P K, Madhan Mohan C, Raut A A, Kumar S, Rai N, Gangwar Soni. (2014). *Laboratory Manual of Biotechnology.* www.bts.org.in.
- Ulmer J B, Donnelly J J, Parker S E, Rhodes G H, Felgner P L, Dwarki V J, Gromkowski S H , Deck R R, DeWitt C M, Friedman A, Hawe L A, Leander K R, Martinez D, Perry H C, Shiver J W, Montgomery D L and Liu M A. (1993). Heterologous protection against influenza by injection of DNA encoding a viral protein. *Science* 259:174561749.
- Wolff J A, Malone R W, Williams P, Chong W, Acsadi G, Jani A and Felgner P L. (1990). Direct gene transfer into mouse muscle in vivo. *Science* 247: 146561468.