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Research Article

EXPRESSION AND IMMUNOGENICITY STUDIES OF A REPLICASE BASED RECOMBINANT PLASMID CONTAINING HBsAg GENE

Chakra Pal Singh[1] and Anant Rai[2]*

[1]Department of Microbiology, Shree Venkateshwara Univeristy, Gajraula, Amroha, UP, India.

[2]Institute of Biotechnology & IT, Mudiya Ahmadnagar, Bareilly-243122, UP, India.

***Corresponding author: raia48@gmail.com**

ABSTRACT

pAlpha-HBsAg recombinant plasmid produced high level of gene expression in HeLa cells as analysed by immunoperoxidae test, It induced satisfactory level of immune response in 4-wwk old mice when given intramuscular single injection 0f 10 µg plasmid DNA since vaccinated mice showed antibody titre of 1/64 while vector alone and healthy mice group did not show any antibody response when tested 28 days after vaccination. It appears it can be tried for use as DNA vaccine.

Key words: Expression, HBV, surface antigen, immunogenicity

INTRODUCTION

Hepatitis B virus infection is a global health concern as it affects over 240 million people worldwide and an estimated 686,000 people die annually as a result of complications of the disease. Several therapeutic strategies are being researched and studied in clinical trials. These strategies include the targeting of the host or viral factors required for viral persistence as well as therapeutic vaccines (Ward *et al*., 2016). Compared with traditional protein-based immunization approaches, DNA immunization is efficient for testing novel immunogen designs, does not require the production or purification of proteins from a pathogen or the use of recombinant protein technology and is effective at generating mAbs against conformation-sensitive targets (Lieu *et al*., 2016). DNA vaccines are ideal for global vaccination because they are inexpensive and easy to manufacture on a large scale and people living in low-income countries can benefit from vaccination (Jorritsma *et al*., 2016).

Administration of replicon RNA vectors has resulted in strong immune responses and generation of neutralizing antibodies in various animal models. Immunization of mice, chicken, pigs and primates with virus-like particles, naked RNA or layered DNA/RNA plasmids has provided protection against challenges with lethal doses of infectious agents and administered tumor cells. Both

prophylactic and therapeutic efficacy has been achieved in cancer immunotherapy. Replicon RNA vectors have also been ued in clinical trials. Overall, immunization with self-replicating RNA viruses provides high transient expression levels of antigens resulting in generation of neutralizing antibody responses and protection against lethal challenges under safe conditions (Lundstrom, 2016). An eukaryotic expression plasmid inserted HBsAg gene (pcDNA3.1-S) was constructed by cloning technique and the accuracy of the construct was confirmed by restriction enzyme digestion and DNA sequencing, then hepatitis B DNA vaccine formulations were prepared by mixing pcDNA3.1-S with various concentration of aluminum phosphate in 0.9% NaCl. It showed that pcDNA3.1-S mixing with aluminum phosphate could increase anti-HBs titers in mice (Liang *et al*., 2004). A reconstructed vector could effectively express short B and T-cell epitope of duck hepatitis B virus, in the culture supernatant as confirmed by dot immunoblot assay. The recombinant single B and T-cell epitope-based DNA vaccine was administrated to mice and could greatly induce specific humoral and CTL response and could be of great use for DNA vaccines (Xu *et al*., 2005). The PR was constructed and then transfected into human hepatocellular carcinoma cell line Hep G2. The antigenicity of PR was examined by EIA, ELISA and immunocytochemical staining. The PR and empty vector pcDNA3.0 were then used respectively to immunize intramuscularly 5 mice, dosage being 100 pg plasmid per mouse. The titers of serum anti-HBs and anti-HBs2 antibodies were detected by ELISA. In vitro experiment showed that mutant HBsAg could bind to anti-HBs antibody. The PR could induce anti-HBs and anti-HBs2 antibody production in immunized mice. But the appearance time of serum anti-HBs2 antibody one or two weeks earlier than that of serum anti-HBs antibody (Ge *et al*., 2003).

Current protein-based vaccines made up of recombinant HBsAg are ineffective in chronic HBV carriers and a significant number of the vaccines do not mount the protective immune response. DNA-based immunization may overcome the deficits of the protein-based immunization and may provide more effective prophylactic and therapeutic outcomes. A recombinant plasmid carrying gene encoding the HBs linked to DNA segment encoding full-length murine interleukin-18, i.e. pcDNA-HBs-IL-18 were constructed, immunogenicity of the DNA construct was carried out in BALB/c mice in comparison with mock, i.e. pcDNA3.1+ and vaccines comprised of pRc/CMV-HBs and pRc/CMV-HBs plus pcDNA-IL-18. All vaccinated mice revealed significant serum anti-HBs IgG response after two intramuscular injections of the vaccines at 28 day interval as compared to the level of mock. Co-administration of pRc/CMV-HBs and pcDNA-IL-18 elicited arbitrarily higher levels of anti-HBs IgG than the levels in mice immunized with pRc/CMV-HBs alone and mice that received pcDNA-HBs-IL-18 although not statistically different (Channarong *et al*., 2007).

MATERIALS AND METHODS

Antiserum against recombinant plasmid

Five healthy 4 week-old Swiss albino mice were immunized with pAlpha-HBsAg recombinant plasmid DNA (Singh and Rai, 2018) intramuscularly in gastrocnemeus muscle of thigh in the hind

leg each with 10 µg DNA. The mice were kept in IBIT Experimental Animal House. Four injections at weekly interval were given. One week after last injection, they were bled from retro-ocular plexus of vein of the eye with a glass Pasteur pipette with blunt end specially made for it. A Pasteur pipette was taken and the tip was cut with a glass cutting foil at a place so that the diameter of the tip is about 1 mm. The tip was ground to make it blunt so that it may not cause any injury. The required volume of blood was collected for serum preparation.

HeLa cell culture

The HeLa cell line was grown in Dulbecco's modified Eagle's medium (DMEM) containing 4mM L-glutamine and 4.5g/L glucose, 1.5g/L, NaHCO3/L with 10% fetal calf serum, pH 7.2-7.4 and incubated at 37C. For sub culturing, removed the medium of the flask and rinsed with trypin-versene solution (TVS). Removed the solution and added an additional 0.5 ml of TVS and allowed the flask to sit at room temperature until the cells detach. Added fresh growth culture medium so as to make 1:4 split ratio and dispensed into new 25 cm^2 flasks. These were incubated at 37ºC in an incubator. Media were changed twice in a week.

Transfection of cells with pAlpha-HBsAg gene

Calcium phosphate mediated transfection of HeLa cells with plasmid DNA in cell culture microtitre plate was used (Rai *et al*., 2016).

Indirect immunoperoxidase test (IPT)

After 72 h of transfection, media from the wells was poured off. Cells were washed twice with 1X 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 mouse anti-HBsAg sera for 2 h at 37^0C, washed three times with PBS and then incubated with rabbit anti-mouse HRP conjugate (Genei, India 1:50 diluted) at 37^0C for 2 h. The cells were again washed thrice with PBS and incubated with 3, 3'-diaminobenzidine (DAB 1mg/ml in PBS with 1 µl/ml H_2O_2) for 5 min at room temperature to develop color. Once the color developed, the cells were washed with PBS, dried in air and were observed under microscope.

Immunogenicity of pAlpha-HBsAg recombinant plasmid

Three groups of 10 Swiss-albino mice of 4 week age were kept in separate mice cages. Pre-vaccination blood was collected for sera preparation. One group was injected with 10 µg pAlpha-HBsAg plasmid DNA (Singh and Rai, 2018) in 0.5ml per mouse in gastrocnemeus muscle of thigh of hind leg (vaccinated group), ten mice were injected with 10 µg pAlpha vector DNA alone per mouse (vector control group), ten mice were kept as healthy control. After 28 days, blood from each group of mice was collected for sera preparation. Sera were kept at -20ºC for further use.

Antibody assay by immunoperoxidase test

HeLa cells in microplates were transfected with 10 µg DNA of recombinant plasmid per well, 10 µg pAlpha vector DNA per well and a group of wells kept as healthy control, based on three wells for each dilution of sera to be tested.72 hours after transfection, sera from mice of different groups were inactivated at 56^0C for 30 min and two-fold dilutions were prepared 1:4 to 1/128

in 96 well micortitre plates by mixing 0.05 ml of sera in 0.05 ml of 0.85% NaCl as diluent. Fifty µl of each sera dilution were transferred to each well using three wells per dilution. These were incubated at 37^{o}C for 2 h after which rabbit anti-mouse HRP conjugate (1:50 dilution) was added. Rest procedure was as described for IPT. The antibody titre was calculated as the reciprocal of the highest dilution that inhibited 50% of the gene expression.

RESULTS AND DISCUSSION

Expression of HBsAg gene in HeLa cells

The HBsAg gene was found to produce high level of gene expression in HeLa cells as revealed by immunoperoxidase test (Figures 1-3). There was an intense color development in gene transfected culture while there was no expression/ color development in vector alone and mock transfected cell cultures.

Immunogenicity of HBsAg gene in mice

All the mice remained healthy throughout the study for one month. The recombinant plasmid DNA was found to induce antibody titre of 1/64 in mice on single dose of vaccination while no antibody response was detected in vector alone and healthy mice groups.

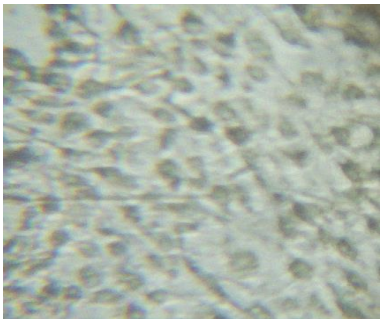

Fig. 1. Mock transfected

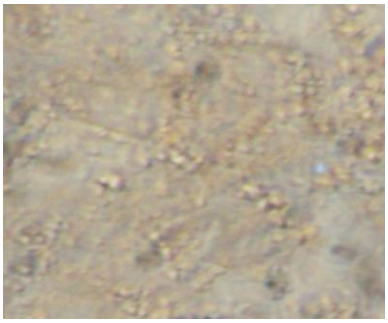

Fig.2. HeLa cells transfected with pAlpha vector

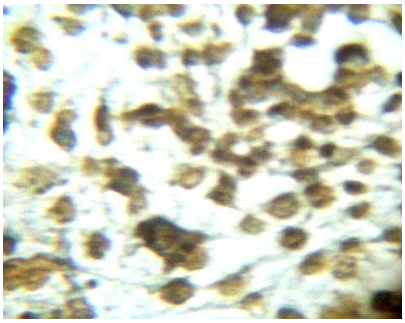

Fig.3. HeLa cells transfected with pAlpha-HBsAg showing positive brown colour.

To combat many emerging infectious diseases, DNA immunization is a unique and powerful approach to production of high-quality monoclonal antibodies against various pathogens. Compared with protein-based immunization approaches, DNA immunization is efficient for testing novel immunogen designs, does not require the production or purification of proteins from a pathogen or the use of recombinant protein technology and is effective at generating mAbs against conformation-sensitive targets (Liu *et al*., 2016). DNA vaccines are ideal candidates for global vaccination purposes because they are inexpensive and easy to manufacture on a large scale such that even people living in low-income countries can benefit from vaccination (Jorritsma *et al*., 2016). An eukaryotic expression plasmid inserted HBsAg gene (pcDNA3.1-S) was constructed by cloning technique and the accuracy of the construct was confirmed by restriction enzyme

digestion and DNA sequencing, then hepatitis B DNA vaccine formulations were prepared by mixing pcDNA3.1-S with various concentration of aluminum phosphate in 0.9% NaCl. It showed that pcDNA3.1-S mixing with aluminum phosphate could increase anti-HBs titers in mice (Liang *et al*., 2004). It is evident by reports of these workers that mammalian expression vectors could be effectively used as DNA vaccine.

CONCLUSION

The pAlpha-HBsAg vector could be used in DNA vaccine trials since it was found to produce high level of gene expression and immunogenic in mice.

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