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DNA VACCINES - PROMISING APPROACH IN PREVENTION OF RABIES

Biochemistry

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ABSTRACT

Rabies is a reemerging and fatal infectious disease in Asia mainly caused by exposure to rabid dogs. The disease is transmitted through the bite of an infected animal, usually from a dog, and can be prevented by the timely administration of rabies immune globulins and post-bite vaccination. Prevention of dog rabies would be the most effective way to stop rabies transmission to humans. However, vaccinating stray dogs in urban and rural areas using conventional vaccines is always difficult and is not cost-effective for use in most areas. This calls for improvement of rabies vaccination strategies. The failure to eliminate dog rabies in most developing countries stresses the necessity of improving rabies control programs by applying new vaccines or new vaccination strategies. There is a need for development of alternative vaccine strategies to overcome the shortcomings of the current conventional vaccines. In spite of great advances in virology there is yet no cure for rabies. Rabies glycoprotein is the major antigen responsible for inducing protective immunity. So it is mostly used in genetic vaccines for producing antigenic protein. DNA vaccination using glycoprotein has immense potential in this regard.

KEYWORDS:

Rabies, DNA vaccine, Glycoprotein, cytokines.

Introduction

Rabies is a serious public health problem in many developing countries, including India. Since there is no effective treatment available after the onset of clinical symptoms, efficient prophylactic immunization is compelling for which a variety of cell culture-derived vaccines are available. **Rupprecht *et al.,* 2002)**. However, the perceived high cost of these products may prohibit their wider use in developing countries. Clearly, new concepts are needed to preserve the record of high potency, purity, safety, efficacy, stability, and economy of rabies vaccine. As such, a number of approaches are being explored for enhanced rabies prevention and control through molecular applications. One of these approaches pertains to utilization of DNA vaccines **(Xiang *et al.,* 1995; Ray *et al.,* 1997; Lodmell *et al.,* 1998; Osorio *et al.,* 1999; Perrin *et al.,* 2000; Lodmell *et al.,* 2001).** The basic mechanism involved in genetic vaccine induced specific immunity is under intense investigation. In brief, the optimized gene construct (preferably a plasmid construct) injected into the host enters the nucleus of transfected local cells. Expression of construct encoded gene is followed by generation of foreign antigens as peptide or protein. These antigens are processed and presented to cells, which work as antigen presenting cells (APCs). These antigen loaded APCs travel to the draining lymph nodes where they present antigenic peptides to T-cells. This interaction provides the necessary signal for cascade of immune response, which includes both humoral and cellular immune response.

Since the pioneering work in early 1990s **(Wolff *et al.,* 1990; Tang *et al.,* 1992; Robinson *et al.,* 1993; Ulmer *et al.,* 1993),** DNA vaccines have demonstrated significant advantages over conventional vaccines. Scientists have successfully applied this technique to develop DNA vaccines against infectious pathogens in many different animal models **(Lagging *et al.,* 1995; Lowrie *et al.,* 1994; Major *et al.,* 1995; Manickan *et al.,* 1995; Michel *et al.,* 1995; Schirmbeck *et al.,* 1995; Ulmer *et al.,* 1993; Wang *et al.,* 1993; Xiang *et al.,* 1994; Yasutomi *et al.,* 1996; Yokoyama *et al.,* 1995);** however, the efficacy of different DNA vaccines has varied widely. With this new technology, recombinant genes encoded by a plasmid expression vector can be delivered and expressed in animal muscle **(Wolff *et al.,* 1990).** Based on this technique, a novel type of vaccine, termed nucleic acid vaccine or DNA vaccine with DNA instead of proteins in the vaccine formulation has been developed **(Donnelly *et al.,* 1995; McDonnell *et al.,* 1996).**

Rabies virus G protein cloned in Mammalian expression vector was shown to protect mice on challenge with 15LD50 of rabies virus CVS 14 days post vaccination **(Rai and Yadav, 2001; Rai *et al.,* 2002; Rai *et al.,* 2005; Gupta *et al.,* 2005, Rai *et al.,* 2006).** Similarly, pre-exposure vaccination with DNA vaccine has been shown to induce protection in mouse by many workers **(Xiang *et al.,* 1994; Ertl *et al.,* 1995; Bahloul *et al.,* 1998; Lodmell *et al.,* 1998; Biswas *et al.,* 2001).** Post exposure vaccination with DNA vaccine against rabies has also produced successful results in mouse **(Lodmell *et al.,* 2001; Bahloul *et al.,* 2003).** Several groups have attempted to induce protective immune response in dogs also by using DNA vaccine with considerable success **(Osorio *et al.,* 1999; Perrin *et al.,* 2000; Lodmell *et al.,* 2003).** A single injection using a jet injector of a rabies DNA vaccine in liquid buffered saline was shown to induce higher level of rabies neutralizing antibodies than multiple injections of comm ercially available cell culture derived vaccine **(Bahloul *et al.,* 2006).** Synthetic rabies virus G protein was shown to be expressed at high level in tobacco leaves and induce protection **(Ashraf *et al.,* 2005).** A recombinant rabies virus carrying two identical glycoprotein (G) genes resulted in overexpression of rabies virus glycoprotein, enhancement of apoptosis and thus, antiviral immune response **(Faber *et al.,* 2002).** It was shown that a DNA vaccine encoding the full-length sequence of the ectodomain plus TD of the mature native RV-G is capable of expressing an 'ideal' immunogen to produce RVNA titers **(Rath *et al.,* 2005).** A bicistronic DNA vaccine against rabies and parvovirus infection of dogs was developed by subcloning rabies glycoprotein and canine parvovirus (CPV) VP2 genes into a bicistronic vector. After characterizing the expression of both the proteins in vitro, the bicistronic DNA vaccine was injected in mice and induced immune response was compared with monocistronic DNA vaccines. This study indicated that bicistronic DNA vaccine can be used in dogs to induce virus neutralizing immune responses against both rabies and CPV **(Patial *et al.,* 2007).**

DNA vaccines and Immune Responses

DNA vaccines have been reported to induce a broad range of immune responses including antibodies, cytotoxic T cells, T-cell proliferation, and protection against challenge with the pathogen **(Lagging *et al.,* 1995; Lowrie *et al.,* 1994; Major *et al.,* 1995; Manickan *et al.,* 1995; Michel *et al.,* 1995; Schirmbeck *et al.,* 1995; Ulmer *et al.,* 1993; Wang *et al.,* 1993; Xiang *et al.,* 1994; Yasutomi *et al.,* 1996; Yokoyama *et al.,* 1995).** Direct injection of the DNA into skeletal muscles results in the synthesis of viral proteins in the host and thus may mimic the action of attenuated vaccines. The in vivo synthesized viral protein can enter both the major histocompatibility complex (MHC) class I and class II antigen-processing pathways and stimulate both arms of immune system, viz., cell mediated immune response and humoral immune response. It has been demonstrated that some DNA vaccines elicit much stronger immune response than others. With one single intramuscular injection of plasmids encoding influenza virus nucleoprotein **(Rhodes *et al.,* 1994) or HBsAg (Michel *et al.,* 1995),** the immunized animals developed long-lasting immunity. The mechanisms underlying the different efficacies of various DNA vaccines have not yet been clearly addressed but presumably are related to the efficacy of transfection, the expression and antigenic

nature of the encoded antigen, and the ability of the protein to be appropriately presented to the immune system. The responsiveness of DNA vaccines to viral antigens may be related to the localization of the expressed protein in the transfected cells. It has been shown that muscle cells are the major targets of the transfected genes after plasmid DNA inoculation **(Acsadi *et al.*, 1991, Ulmer *et al.*, 1993, Wolff *et al.*, 1990).** However, myoblasts and myocytes express only low levels of MHC class I determinants and appear not to express MHC class II and the accessory costimulatory molecules, such as B7-1/B7-2 **(Hohlfeld and Engel, 1994),** which are important for Th-cell activation **(June *et al.*, 1994; Bretscher, 1992).** Thus, it is unlikely that the transfected myoblasts/myocytes serve as antigen-presenting cells to stimulate CD4 Th lymphocytes. The priming T cells may involve the release of the encoded antigen from the transfected muscle cells to the draining lymph nodes where the antigens could be recognized by B cells and could be processed by "professional" antigen-presenting cells, such as macrophages and Dendritic cells, for presentation to T cells with appropriate costimulation. A possible mechanism for the membrane-bound protein to induce immune responses is that due to the inoculation of the plasmid DNA, some damaged muscle fibers release a small quantity of antigens to reach the draining lymph nodes. There, the antigens are recognized by B cells and other antigen-presenting cells and function in antigen processing and presentation to activate naïve Th cells. Thereafter, the prolonged cell surface expression of antigens and the MHC class I/peptide complex display may serve to boost activated B cells and T cells, respectively, which are considerably less dependent on accessory cell costimulation than are naive cells.

Immunomodulation

Plasmid vectors containing a CpG dinucleotide motif elicited much stronger humoral and cellular immune responses to the encoded antigens than did vectors, which did not contain this sequence **(Sato *et al.*, 1996).** The adjuvant activity of the CpG motif was closely related to its ability to elicit a rapid cytokine release from the transfected cells. The direct evidence that cytokines could influence the efficacy of DNA vaccination was shown by **Irvine *et al.* (1996).** They showed that in a mouse tumor model, when recombinant IL-2, IL-6, IL-7, or IL-12 was added following administration of DNA encoding a tumor-associated antigen, the number of established metastases was significantly reduced compared with that in mice treated with DNA only. However, because of the pleiotropic nature of cytokines, the systemic administration of cytokines at the therapeutic levels produced not only the intended immune induction but also undesirable nonspecific responses. The cytokines, as immunological adjuvants, can enhance various immune responses when administered during the development of an immune response to a particular antigen **(Dong and Ho, 1995).** Interleukin-2 (IL-2) is perhaps the most extensively studied of all cytokine adjuvants. The adjuvant effects of cytokines were also observed in the case of genetic immunization. A sustained but low level of cytokines delivered to tissues of immune interactions may reduce the toxicity of these pleiotropic compounds while improving their therapeutic and practical value in providing vaccine adjuvant effects. **Raz *et al.* (1993)** reported that intramuscular injections of plasmids encoding IL-2, IL-4, or type b1 transforming growth factor successfully modulate immune responses to transferrin delivered at a separate site. Plasmid encoding IL-2 enhanced both cellular and humoral immune responses, while plasmid encoding type b1 transforming growth factor depressed the anti-transferrin response. **Pinto *et al.* (2003)** have shown that chemokines given as genetic adjuvants can augment the transgene product specific immune response to a DNA vaccine. Chemokines not only function as chemoattractants but also have immunomodulatory activities on cells of the innate and adaptive immune system.

Advancement in DNA vaccines

In order to improve the efficacy of DNA vaccine, several attempts have been made from using different methods of delivery to different kinds of adjuvants. **Arya *et al* (2016)** proposed an innovative approach for mass vaccination of dog using a dissolving microneedle patch. It was also shown that vaccine was stable upon formulation and storage for at least 3weeks at 4°C in a microneedle patch. For vaccination, the patches were applied to the inner ear by hand without an applicator. It has been proved that microneedle patches are at least as immunogenic as intramuscular injection at the same dose, as demonstrated by similar serum neutralizing antibody titers. **Ullas *et al* (2014)** have tested the Immunogenicity and efficacy of a plasmid DNA rabies vaccine incorporating Myd88 as a genetic adjuvant. Myeloid differentiation factor 88 (Myd88), a ubiquitous Toll-like receptor adaptor molecule, has been reported to play important roles in B cell responses to infections and vaccination. Genetic adjuvanting with Myd88 enhanced the RVNA responses by 3- and 2-folds, following intramuscular and intradermal immunization, respectively.and protective efficacy of a plasmid DNA rabies vaccine upto 80%. **Garg *et al*(2017)** demonstrated that alum adjuvanted rabies DNA vaccine pgp.LAMP-1confers 80% protection against lethal 50 LD50 rabies challenge virus standard strain. This DNA vaccine conferred 60% protection to BALB/c mice against 20 LD_{50} rabies challenge virus standard (CVS) strain challenge. However upon supplementation this DNA vaccine with Emulsigen-D, the vaccine formulation conferred complete protection against lethal challenge. To assess the feasibility of this vaccine formulation for human use, it was tested along with other FDA approved adjuvants, namely, Alum, Immuvac, Montanide ISA720 VG. Enhanced immune response correlated with high IgG antibody titer, Th2 biased response with a high level of rabies virus neutralizing antibodies (RVNAs) and IgG1/IgG2a ratio >1, observed upon alum supplementation of the rabiesDNA vaccine. The total IgG antibody titer was 2IU/ml and total rabies virus neutralizing antibody titer was observed to be 4IU/ml which is eight times higher than the minimum protective titer recommended by WHO. Furthermore, it conferred 80% protection against challenge with 50 LD_{50} of the rabies CVS strain.

The DNA vaccine is safe, technically simple and elicits immune response against various dreaded pathogens. Further this technique affects both humoral and cell-mediated immunity (CMI). Several clinical trials of DNA vaccines against HIV, influenza, malaria, hepatitis B and herpes virus and in cancer therapy are under evaluation. In 2005, the U.S. Department of Agriculture (USDA) licensed first DNA vaccine against West Nile virus for use in horses and expressed favourable recommendations towards this technology for use in future. However DNA vaccines are still far from perfections and still need to be optimized to get 100% protection.

Conclusion

The DNA vaccines from initial demonstration of their efficacy have rapidly shown progress in clinical trials. By the use of cytokine genes, distinct and newly discovered adjuvants, delivery devices, and mixed modality approaches hold great promise for new vaccines and immunotherapeutics. In parallel they are also being utilized as research tools to study genetic information arisen from the field of genomics. The fundamental simplicity of DNA vaccines combined with the understanding of immune mechanisms and molecular biological manipulations will provide a platform useful for a understanding and possible treatment of diseases including rabies.

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