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Commentary

DNA vaccines: getting closer to becoming a reality

Vaccination, now a century-old technique, essentially involves stimulating the immune system with an infectious agent or components thereof, modified in such a manner that no harm comes to the immunized person[1]. Importantly, upon subsequent exposure to the same pathogen, protection is conferred to the individual by the immune memory. This is the basic essence of vaccination. Moreover, while individuals are protected against development of disease; populations are protected against the spread of the disease-causing agent. In a remarkable feat, eradication of the dreadful Small pox virus from earth was achieved through sustained vaccination, and polio is now on its way out. It is estimated that of the approximately three decades added to average human life span in the past century, 10-15 years have resulted from vaccination alone[2].

Traditional vaccines have either been killed/inactivated or live-attenuated, which were usually developed following an empirical approach. As our knowledge on the pathogens and the mechanisms of immune responses advanced, the empirical approach to vaccine development was replaced by a more rational one. It became evident that inoculation of one or more proteins of the pathogen, rather than the entire pathogen was sufficient to evoke a protective immune response. This led to the development of subunit vaccines. With the advent of recombinant DNA technology, subunit vaccines are now produced using molecular cloning techniques. One step further takes us to the realm of DNA vaccines, a radically new approach to vaccination[3]. Here, a plasmid DNA encoding the antigen of interest is used for vaccination, so that the antigen is synthesized *de novo*, following the administration of the DNA. Since the first demonstration of the immune response generated to the antigen encoded by the DNA vaccine around two decades ago[4], a lot of progress has been made in understanding the basic biology behind this apparently simple technique, and much technological advancements have been made to enhance the immune potency.

DNA vaccination offers a number of potential advantages over traditional vaccination, including stimulation of both B- and T-cell immunity, improved thermostability precluding the need for a cold chain, absence of any infectious agents in the vaccine preparation, and the relative ease of up-scaling for large-scale manufacturing[5]. The proofs-of-concept have been amply demonstrated in various animal models, both small and large, including rodents and non-human primates, using a plethora of antigens. Human clinical trials have also been carried out with DNA vaccines for diseases like hepatitis B, HIV/AIDS and malaria to name a few; albeit this category of vaccine is yet to be licensed for human use[6].

The paper by Dinesh Kumar and colleagues[7] in this issue on DNA rabies vaccine and combination rabies vaccine in Rhesus monkeys (*Macaca mulatta*) represents a pioneering work from India in the area of DNA vaccines. This is perhaps the first pre-clinical toxicological evaluation of a DNA vaccine in non-human primates in India. Importantly, the vaccine under study is the first indigenously developed DNA rabies vaccine (DRV). The combination rabies vaccine (CRV) is a formulation of the DRV along with a cell culture-derived inactivated rabies virus vaccine. The combination rabies vaccine (CRV) is intended to increase the potency of the DRV, which was found to confer only suboptimal levels of protection in murine rabies virus challenge model[8,9]. This formulation was more potent than the DRV and elicited antibodies much more quickly in immunized mice and cattle[10]. The vaccine is intended for clinical use in humans as well as in veterinary practice by intramuscular route, subsequent to its pre-clinical safety evaluation in a higher animal model.

As per the regulatory requirements, the study was designed to evaluate toxicity in acute, sub-chronic and chronic dosing schedules. Three dosing levels, including therapeutic, average and highest-dose, were used. The authors have addressed all the important issues that are likely to be required to be addressed for obtaining regulatory clearance to proceed for human clinical trials. The safety of both the DRV and CRV has been previously confirmed in murine model[11] and corroborated by the present study in non-human primates. All the relevant parameters, including physical, physiological, clinical, haematological and histological profiles of the target organs were evaluated, and the vaccine was found to be safe. As the test compound is a novel product, its complete safety was also demonstrated with special reference to immunogenicity/immunotoxicity by conducting tier-III investigations that showed the absence of anti dsDNA antibody as well as anti-nuclear antibody in immunized animals. The present pre-clinical evaluation of the vaccine is, thus, both comprehensive and exhaustive and should suffice for obtaining regulatory clearance for human clinical trials. However, the presence of residual plasmid DNA, even though in traces of less than a femtogram, at the injection site of one of the immunized monkeys warrants a careful follow up.

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