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STRATEGIES TO ENHANCE THE POTENCY OF HIV-1 DNA VACCINES

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ABSTRACT

Despite 30 years of intense research on HIV/AIDS, we have yet to arrive at a prophylactic vaccine that confers complete protection. This mission is complicated by the virus's vast genetic variability and its ability to mask the targets for neutralizing antibodies. In addition, HIV infects a subset of immune cells that normally coordinate the immune system and integrates its genome into the DNA of the infected cell. The failure of early HIV vaccine candidates based on classical vaccine strategies has underscored the importance of exploring alternative vaccine approaches, including DNA vaccines. These vaccines are capable of inducing broad cell-mediated and humoral immune responses and their potential is indicated by the licensing of DNA vaccines for veterinary use and by the induction of protection against infectious diseases in animal models. Still, further efforts are needed to ultimately make this approach efficacious in humans. This thesis describes means of enhancing the potency of DNA vaccines for HIV-1, such as by optimization of the gene insert, use of delivery devices and combinations of vaccine candidates.

In one project, we constructed DNA vaccines expressing different variants of the HIV-1 protease and determined that both *in vitro* expression and immunogenicity of the encoded protein in mice were drastically enhanced when a point mutation was introduced in the active site of the protease enzyme, rendering it inactive. We thus discovered a means of enhancing the immunogenicity of HIV-1 protease. Another project was designed to establish an immunization protocol for electroporation (EP)-mediated intradermal DNA vaccine delivery. We showed that a straightforward protocol, using repeated intradermal EP immunizations with a rather short immunization interval, induced strong and long-lived immune responses. A novel FluoroSpot assay detecting vaccine-specific secretion of gamma interferon (IFN-γ) and/or interleukin-2 (IL-2) was shown to possess the advantages of both ELISpot and intracellular staining. Further evidence supporting the use of EP for the delivery of DNA vaccines was obtained in a study where a combination of jet injection and EP, but not needle plus EP or jet injection alone, was able to overcome dose restrictions of DNA vaccination and induce higher antibody and cytotoxic T cell responses when the DNA dose was increased to a considerably higher level. This shows that two optimized DNA vaccine delivery devices can act together to overcome dose limitations of a plasmid DNA vaccine.

Experiments evaluating the combined effect of different vaccine modalities were conducted. In one study, two plasmids included in the clinically evaluated HIVIS multigene/multisubtype HIV vaccine encoding Env and Rev were combined with the Auxo-GTU-MultiHIV multigene DNA vaccine that is primarily designed to induce cell-mediated immune responses. Immunization of mice revealed that strong immune responses against the two vaccine modalities were retained, with only a slight reduction of cellular immune responses when the vaccines were administered to the same mice. Moreover, heterologus prime-boost immunizations of mice with DNA, recombinant vaccinia vector (MVA-CMDR) and recombinant protein (rgp140C) induced potent cell-mediated and humoral immune responses and demonstrated the importance of including DNA priming immunizations. These attempts to enhance the potency of DNA vaccines will potentially contribute to the understanding of how to construct, deliver and compose the next generation of DNA vaccines against HIV as well as other infectious diseases and cancers.

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