commercially available nonsynthetic vaccines in inducing protective levels of antibodies in infants.

Virus-Like Particles

VLPs are structures resembling a virus but empty of nucleic acids, which are derived from self-assembling subunits of virus structural antigens. VLP vaccines combine many of the advantages of whole-virus vaccines (induction of strong immune responses) and recombinant subunit vaccines (relative simplicity in manufacturing a safe vaccine). Commercialized VLP-based vaccines have been successful in protecting humans from HBV and HPV infections. These vaccines have excellent safety profiles, are highly effective, and induce long-lasting antibody responses. VLP vaccines are currently being explored for their potential to combat other infectious diseases and cancer. Many VLPs, including HPV VLPs, do not require coadministration of exogenous adjuvants to induce strong antibody responses. In addition, VLPs efficiently induce T-cell responses through interactions with antigen-presenting cells, particularly dendritic cells.

VLPs are routinely expressed and produced from yeast, mammalian, and insect cells (the baculovirus expression system). VLPs designed for mucosal vaccination can also be efficiently produced in gut bacteria such as highly attenuated *Salmonella* or *Lactobacillus* strains and even in plants for oral inoculation (18).

VLPs have also been exploited as molecular scaffolds for heterologous antigen presentation. Many different VLPs have been adapted for this purpose by incorporating heterologous epitopes into already well-characterized surface loops of virus antigens, generating chimeric particles. Since multiepitope vaccines have been shown to be more successful in inducing broad immune responses, a second generation of VLPs has been designed incorporating epitopes from more than one antigen of the same viral agent or antigens for different pathogens in a combined vaccine. A successful example of this is the synergistic effect resulting from vaccination with a combined CombiHIVvac vaccine, which incorporates B-cell and T-cell epitopes from Env and Gag proteins in a multicomponent VLP containing a DNA vaccine encoding multiple immunogens of HIV-1 (19). The level of antibodies induced by immunization with any of the immunogens was significantly lower compared with that induced by the combined vaccine.

Lessons learned from combating well-known viruses like HBV, HPV, or HIV are constantly being carried over to newly emerging and less intensively studied viral diseases, for which VLP-based strategies might serve as attractive first-step tools to develop protective vaccines. Furthermore, the VLP-based technological platform is being exploited for innovative new vaccines directed against nontraditional targets such as self-antigens involved in chronic diseases (e.g., rheumatoid arthritis) or for vaccines toward a better standard of living such as the antismoking vaccine that targets nicotine. Through chemical conjugation of nicotine to a VLP-based vaccine, it was demonstrated that, when a sufficient antibody level is achieved, continuous abstinence rates can be significantly increased by vaccination (20).

Replicating and Nonreplicating Vectors

Historically, live attenuated, replicating vaccines, such as measles, mumps, rubella, polio, vaccinia, and yellow fever, rather than inactivated preparations, have provided the most effective protection against viral infection and disease. Notably, these vaccines elicit essentially lifelong protective immunity (21). The idea of using viruses as gene delivery vehicles to combat diseases has been an obvious next step. The failed efforts to develop effective vaccines against AIDS and malaria led to the development of a wide range of innovative viral vectors that are able to efficiently deliver antigens and induce immune responses (22). A broad spectrum of replicating and nonreplicating vectors are available. A variety of attenuated viruses have been employed as vectors including vaccinia and other pox viruses, adenovirus, and single-stranded RNA virus replicon vectors such as alphaviruses, coronaviruses, picornaviruses, flaviviruses, influenza viruses, rhabdoviruses, and paramyxoviruses. Choice of an appropriate vector for use in the development of a vaccine depends on the biology of the infectious agent targeted, as well as multiple other factors. These include whether the vaccine is intended to prevent infection or to boost immunity in already infected individuals, prior exposure of the target population to the vector, safety considerations, the number and size of gene inserts needed, and suitability for large-scale manufacturing and compliance with regulatory requirements.

Studies in animal models suggest that each viral vector is unique in its ability to induce humoral and/or cellular immune responses. Most vectored vaccines are designed to elicit cellular immune responses. In prime-boost regimens, vector priming followed by a booster inoculation with a protein antigen can induce broad and potent antibodies. Alternatively, some viral vectors such as alphavirus can be engineered to elicit potent antibody responses (23) and might prove to be a useful alternative (21).

Use of recombinant replication-proficient and nonreplicating vectors will face extensive preclinical testing and will possibly have to meet stringent regulatory requirements. However, some of these vectors may benefit from the profound industrial and clinical experience of the parent vaccine (22). For instance, among the promising vectors is the measles virus, with a long-standing safety and efficacy record. The measles vaccine induces strong cellular and humoral immune responses after a single injection, and it is likely that a multivalent vaccine could be produced on a large scale and at low costs similar to the parental vaccine, making it an attractive model platform for the development of live attenuated, recombinant vaccines. However, even in this case, the modified recombinant vectors will need to be carefully tested for safety to make sure that the modification has not introduced unexpected properties.

DNA Vaccines

The discovery in the early 1990s of DNA immunization radically changed accepted views of the nature of a vaccine. Instead of delivering an antigen per se, genetic material that encodes a specific antigen is delivered into mammalian cells, directing expression of the antigen by the cell itself, essentially working from the inside out. Numerous viral, bacterial, and parasitic antigens have been delivered by DNA vaccines, with varying success rates to date. The high expectations associated with DNA vaccination, as a result of promising data obtained in animals, were somewhat tempered by disappointing early results when DNA was tested as a vaccine in humans. A recognized limitation of DNA vaccines is their limited capacity to induce good virus-neutralizing antibody responses. New