DNA VACCINES

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Traditionally, protection against infectious diseases has relied on the use of attenuated or killed vaccines. However, many such vaccines are inadequate for reason of efficacy, safety, and cost effectiveness. Live-attenuated vaccines may be immunosuppressive, cause disease if not attenuated sufficiently, or provide limited immunity if too much attenuated. A major concern regarding the use of live vaccines is the possibility of outgrowth of more virulent organisms. Killed vaccines are often unable to generate protective levels of immunity for reasons of Ag load and loss of important epitopes during inactivation. In addition, they are frequently inconvenient, given that repeated immunization is often necessary to achieve effective levels of immunity. Further, because killed viral vaccines do not provide endogenously synthesized proteins, they are in general unable to induce cytotoxic T cells, possibly a required component of a truly effective vaccine. Subunit vaccines are generally safe but costly and poorly immunogenic. Live recombinant vaccine vectors are effective, but their repeated use in the same host may be limited by vector immunity; furthermore, they are subject to reversion events and can cause disease or death in immunocompromised hosts. There is a need for better vaccines, and recently DNA vaccines have been developed (1).

DNA vaccine technology offers a promising new alternative to conventional vaccine delivery systems. DNA vaccines are eukaryotic expression vectors that contain the gene encoding the antigen of interest. Within intramuscular injection, plasmid DNA enters muscle cells, direct expression of the plasmid-encoded antigen, and an immune response to the antigen is induced. DNA vaccines have been studied in many systems, including influenza, human immunodeficiency virus (HIV), rabies, herpes virus, and hepatitis B, and can induce long-lasting humoral and cellular immune responses. DNA vaccines are easily constructed by techniques common to all molecular biology laboratories, should be inexpensive to produce, and should not require refrigeration; all essential attributes for a vaccine intended to be used in the developing world. Another potentially useful feature of DNA vaccines is that they can be designed to express more than one antigen simultaneously, either by encoding multiple antigens (or epitopes) on a single plasmid or by immunizing with a mixture of plasmids, each encoding a single antigen (2).

Preclinical efficacy of DNA vaccines

Influenza

The first demonstration of DNA vaccine efficacy in an animal model was using the influenza virus, which continues to provide a useful system with which to characterize immune responses to DNA vaccines. Influenza is one of the infectious disease targets for a DNA vaccine currently being investigated in human clinical trials. One rationale for efforts to develop an influenza DNA vaccine, despite the availability of widely utilized existing influenza vaccines, is that current vaccines are effective only in a strain-specific manner. Mutations in influenza virus strains mean that frequent re-evaluation and reformulation of the vaccine is necessary. DNA vaccines offer the advantage of stimulating the generation of cytotoxic T lymphocytes (CTLs) against epitopes from a conserved protein of the virus. A combination DNA vaccine containing plasmids encoding both internal and coat proteins of the virus was prepared in a mixture to mimic the proteins present in the whole virus vaccine. Vaccination of nonhuman primates generated titers of hemagglutination inhibiting

antibodies (used as surrogates for neutralizing antibodies) as high as a or higher than those generated by the full human doses of whole inactivated and split inactivated virus vaccines, respectively. Ferrets immunized with the DNA vaccine cocktail shed less virus in nasal washings after challenge with a drifted (i.e. antigenically different) strain of virus than did control animals immunized with the analogous commercially licensed whole inactivated virus vaccine (3, 4, 5).

Malaria

Another model in which a combination DNA vaccine was recently employed is malaria.

In this disease there are two problems with vaccine studies. One, because it is unlikely that a vaccine directed against a single antigen expressed during one stage of the life cycle will remove all parasites, a vaccine will probably need to target several antigens from different life-cycle stages. A DNA vaccine against malaria could be designed to express antigens from sprozoite, exoerythrocytic and erytrocytic stages and therefore raise immun responses against multiple life cycle stages. Second, in malaria there are relatively few target epitopes different parasite isolates and the variants often do not crossreact. Thus, to elicit effective immunity against multiple wild isolates with variant epitopes, immunization with a vaccine with coding many antigens should be required (2).

Other viral and bacterial disease models

Also important for bringing DNA vaccine technology closer to clinical application was the continued expansion of the breadth of preclinical infectious and noninfectious disease targets successfully tested in animal models. These included additional viral targets, as well as parasitic diseases, bacterial diseases, and cancer (against which the first clinical trials for DNA vaccines were initiated during 1995). Joining the viral disease targets for which immune responses and/or protectionhad already been demonstrated (such as influenza, bovine herpesvirus, hepatitis B, HIV and rabies) were hepatitis C virus, herpes simplex virus, papillomavirus, lymphocytic choriomeningitis virus and flavivirus. The list of parasitic diseases for which preclinical efficacy has been demonstrated was expanded from malaria and leishmaniasis to include schistosomiasis (3, 6).

It is possible that bacterial diseases were not the initial target for DNA vaccines because a number of protective bacterial antigens are not proteinaceous (e.g. polysaccharides) and because less is known about the protective antigens of bacteria compared with viruses (i.e. those antigens that induce a protective immune response). For at least some bacterial targets, however, not only are proteins key antigens, but the types of cellular responses induced by DNA vaccines may be important elements of an effective vaccine. An example of such a bacterial target is Mycobacterium tuberculosis; the cellular responses against its proteins appear to play key roles in protective immunity. Recent work has shown that tuberculosis DNA vaccines induce CTL and helper T cell responses of the Th1-like phenotype, as measured in vitro upon restimulation of spleen cells with antigen (3, 6, 7).

Cancer immunotherapy

DNA-based immunization is an attractive nonviral alternative for cancer immunotherapy. DNA vaccination is accomplished by the expression of inoculated bacterial plasmid DNA encoding the foreign gene of interest accompanied by a mammalian promoter/enhancer, and other sequences such as Kozakís consensus sequence and leader sequences that enable the gene to be expressed within mammalian cells utilizing host machinery. In this report, a hand-held helium-powered device was used to achieve the direct intracellular delivery of DNA-coated gold particles to the epidermis. Following delivery, the DNA redissolves in the apueous environment of the cytoplasm or nucleus and is then available for expression. Alternatively, skeletal muscle cells have demonstrated the ability to take up and express DNA for approximately the lifetime of the mouse without any specific delivery system. However, epidermal gene gun immunization of

DNA may be more efficient than intramuscular immunization at eliciting similar immune responses (8).

Both DNA-based approaches have been shown to successfully induce both humoral and cellular immunity in many Ag systems . In cancer immunothrepy, plasmid constructs encoding either the full length cDNA for carcinoembryonic Ag (CEA) or HIV-I envelope protein, gp16, have been shown to protect mice from subsequent challenge with syngeneic tumors expressing these model Ags (8).

DNA delivery

DNA vaccines could be modified by the use of a delivery system, such as liposomes or polymers that can compact DNA and enhance cellular uptake, or the inclusion of peptides or proteins that can facilitate intracellular targeting of DNA to the cytoplasm and nucleus. In addition, DNA vaccines may be targeted to specific tissues such as mucosal sites for the induction of mucosal immune responses. To this end. Sizemore contains an asd (aspartic/semialdehyde dehydrogenase) mutation that does not interfere with the ability of the organism to invade cells but which causes it to burst open inside the cell, thereby releasing expression plasmids into the cytoplasm. Recombinant Shigella containing plasmids expressing galactrosidase under the control of the human cytomegalovirus (CMV) early promoter and enhancer are not themselves able to express galactosidase but can direct expression of 3galactosidase in cell cultures, in the guinea pig eye, and in the mouse lung. Mice immunized in this manner produced cellular immune responses and high levels of specific antibodies. HIV and malaria genes have also been expressed in this system. These findings open up to possibility for the relatively inexpensive oral delivery of functional DNA with the potential for manipulation of the local immune system as well as for production of systemic responses (3).

Expression library immunization

The identification of the protective antigens of a pathogen is a laborious and sometimes problematic process. This is particularly true for protection that requires cellular immunity, since certain types of vaccines (e.g. subunit proteins or whole inactivated viruses) do not generally induce CTLs. Furthermore, resting specific antigens requires that they be available in a purified form. With DNA vaccines, CTLs are readily induced and one needs only to have the gene encoding the antigen. The process of vaccine antigen discovery may be simplified by a recent and exciting application of DNA vaccine technology. Barry et al. developed a method for testing mixtures of DNA plasmids containing fragments of the genome of a pathogen for protective efficacy (termed expression library immunization, or ELI). In their example, vaccinations with mixtures containing 3000 distinct plasmids from a Mycoplasma pulmonis DNA library were shown to confer protection in a mouse challenge model, indicating that at least one of the plasmids encoded a protective antigen. By successive fractionation and testing of these mixtures it may be possible to identify the protective plasmids, although to date such fractionation and identification has not been reported. One of the potential drawbacks of the technique in addition to the potential masking of epitopes by immune interference is that, because fragments of the genome are used, many of the plasmids will not encode a relevant protein. This problem can be overcome by cloning open reading frames into the expression library and, with the burgeoning filed of genomics, this sequence information is rapidly becoming available for many pathogens. Such approaches may greatly facilitate the identification of vaccine antigens (3).

Conclusions

The use of DNA vaccination has grown substantially in the eight years since it was first demonstrated that DNA could confer protective immunity. Recent important advances have been made in several aeas of DNA vaccines. These include an expansion of the targets for DNA vaccine development, a greater understanding of some of the underlying mechanisms involved in the induction of immune responses, the beginnings of alternative DNA delivery vehicles that can target mucosal immune sites, the application of DNA vaccine technology to the

discovery of protective antigens and the human clinical trials. The success of DNA vaccines will be predicated on their effectiveness in humans.

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