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Review Article

Delivery Systems for Gene-based Vaccines

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Abstract

Along with the elucidation of the role of cytotoxic T lymphocytes in the immune responses against a number of pathogens and cancer, and with the increased understanding of the cellular processing mechanisms of antigens for generation of these cells, has come an increased focus on vaccines that can generate cellular immunity along with antibodies. Promising approaches based on the delivery of genes, either as plasmid DNA or by viral vectors, have been extensively evaluated pre-clinically and in early-phase clinical trials. Although the first generation of DNA plasmid vaccines were broadly effective in animal disease models, early clinical immunogenicity pointed towards the need for increased potency. This manuscript reviews recent developments for gene-based vaccines, specifically, new approaches for formulating and delivering plasmid DNA and alphaviral replicon vectors, all of which have resulted in increased potency of gene-based vaccines.

Introduction

Increased understanding of the role of cellular immune responses, specifically cytolytic T-cell (CTL) responses, in the control or prevention of viral and parasitic infections and cancer has led to increased efforts to design vaccines that elicit these CD8+ T cells. The different pathways by which proteins are processed and presented by major histocompatibility complex (MHC) Class I and Class II molecules, leading to priming of cytolytic CD8+ and helper CD4+ T cells, respectively, largely have been elucidated. In general, for a protein antigen to be degraded into peptides that bind MHC Class I molecules, the protein must be present in the cytoplasm of an antigen-presenting cell. Proteins that are exogenous to a cell are internalized and degraded within endolysosomes and, thus, preferentially encounter MHC Class II molecules. In the cytosol, a specialized system exists for degrading proteins and transporting the resulting peptides into the endoplasmic reticulum for binding to nascent MHC Class I molecules. This complex is then transported to the surface of the antigen-presenting cell (APC). In the presence of certain cell surface proteins referred to as co-stimulatory molecules, engagement between the APC and naïve CD8+ T cells results in the priming of antigen-specific cytolytic T lymphocytes.

Because of the challenges related to delivering proteins into the cytosol of APCs, many vaccine strategies focus, instead, on the delivery of genes encoding the desired proteins. In this manner, the antigen simply can be produced in the cytosol and have direct access to antigen presentation machinery. Various gene delivery systems include intracellular bacteria (such as *Mycobacterium tuberculosis, Listeria monocytogenes, Salmonella typhi, and Shigella flexneri*), viruses (such as vaccinia, adenovirus, and avipox), and

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plasmid DNA. Delivery of DNA by live vectors is quite efficient, relative to naked DNA vaccines. However, replication-incompetent or attenuated strains are needed to avoid complications from the pathogenic organisms. Other issues, such as cellular targeting and immune responses against viral proteins, also must be considered.

Until the 1980's, plasmid DNA was not thought capable of directly transducing cells in vivo. Reports by Dubensky, et al. (1) and Benvenisty and Reshef (2) demonstrated that direct inoculation of rodents with calcium phosphate-precipitated plasmid DNA resulted in expression in vivo. Subsequently, injection of naked plasmid DNA was shown to transfect muscle cells in vivo (3). Proof of concept for the delivery of plasmid as a means of vaccination was demonstrated by the induction of antibodies (4) and CTL (5). Importantly, the immune responses induced could protect animals against death following a lethal viral (influenza) challenge (5), thereby, providing a potential method for delivering genes for vaccines that did not require a live pathogen. The disease models in which DNA vaccines have been shown to be effective (ranging from infectious disease to cancer to auto-immune disease) are too numerous to list, but are reviewed in Donnelly, et al. (6). However, it has become apparent that the potency of DNA vaccines needs to be increased if this technology is to be effective in large animals, including humans. This review will focus on promising approaches to augment the efficacy of DNA vaccines, namely new delivery systems and alphavirus replicons, and possible synergy between the two.

DNA Vaccines

DNA Vaccine Expression Vectors

There are several possible distinct approaches to increasing the potency of DNA vaccines. First, modification of the plasmid DNA vector to increase expression levels or to target antigen expression to specific intracellular or extracellular locations has resulted in increased immunogenicity in vivo. For example, changing the nucleotide sequence of certain genes to better reflect preferential codon usage in mammalian cells can result in markedly higher levels of expression in eukaryotic cells in vitro (7) and, when incorporated into a DNA vaccine vector, can increase immunogenicity substantially (8–10). Incremental increases in expression levels can also be achieved by modification of promoter and transcription terminator regions of the vector (11), but these increases are modest, compared with those achieved by the promoter/enhancer of cytomegalovirus (CMV). This promoter offers relatively high levels of expression in a variety of cell types and, hence, is widely used in DNA vaccines. However, it is also susceptible to down-regulation by cytokines, such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α (12). Therefore, it is possible that locally high concentrations of these cytokines induced by DNA vaccines in situ may limit the duration and magnitude of antigen expression. This conceivably could result from the action of immunostimulatory (CpG) motifs within the bacterially derived plasmid on macrophages, natural killer cells, or dendritic cells (13), or from the antigen-specific responses of T cells. For this reason, it may be advisable to consider alternative promoters that function in various cell types, including APCs, and that are not adversely affected by cytokines.

In addition to expression levels, the intracellular and extracellular sites to which the DNA vaccine antigens are targeted may affect the quality and quantity of the immune response. In general, DNA vaccines encoding secreted antigens are more immunogenic for both antibodies and cell-mediated immunity (CMI) than are non-secreted antigens (14,15). In some cases, though, anchoring of antigens on the surface of cells, by virtue of a membrane-spanning domain, results in higher levels of antibody responses (16), possibly due to a longer half-life of the protein. Preferential induction of CTL responses can be elicited by targeting antigen for rapid degradation by the proteasome, as achieved by expressing a fusion protein with ubiquitin (17,18). However, this approach does not always succeed (19,20) and, thus, may be dependent upon the antigen. The use of DNA encoding fusion proteins containing ligands that target cell surface receptors of APCs also has resulted in enhanced immunogenicity. For example, fusions with CTLA4, L-selectin (21), and chemokines (22) yielded higher antibody and CMI responses, presumably due to cross-priming by targeting of antigen to B7, CD34, and chemokine receptors, respectively.

DNA Vaccine Adjuvants

A second general approach to improving DNA vaccines is through the use of adjuvants,

which can include proteins, compounds, or DNA plasmids encoding immunologically active proteins, such as cytokines, chemokines, and costimulatory molecules. The specific examples are too numerous to list here, but are reviewed elsewhere (23). It is likely that expressed cytokines provide additional T- and B-cell helper responses; whereas, expression of chemokines may result in attraction and/or activation of APCs. With respect to the effect of costimulatory molecules, it has been postulated that expression of these proteins in non-APCs may confer transient APC function to these cells (24). Simple mixtures of DNA vaccines with adjuvants are sometimes effective, but appropriate formulation may be required. For example, certain aluminum salts (such as aluminum phosphate), when mixed with DNA vaccines, enhance antibody responses (25); whereas, others (such as aluminum hydroxide) conversely inhibit responses as a consequence of electrostatic interaction between the negatively charged DNA and positively charged adjuvant. This detrimental effect can be overcome with appropriate formulation to prevent such binding.

The inherent adjuvant effects of unmethylated CpG motifs within DNA vaccines likely contribute to their effectiveness, since:

1. the addition of non-coding plasmid DNA can increase immunogenicity of antigen-encoding DNA (6);
2. methylation of plasmid DNA reduces immunogenicity (26); and
3. in some cases, cloning of additional CpG motifs in DNA vaccines can increase potency (27,28).

The potential simplicity of utilizing CpG effects has led many investigators to test modified vectors and/or mixtures of CpG-containing oligonucleotides with DNA vaccines with mixed success. Although some reports have shown modest enhancement (26–28), many attempts have resulted in little effect, or reduced effectiveness (29). In the case of CpG motifs within the vector, the flanking nucleotide sequence is likely to be critical. Also, the presence of neutralizing motifs that can interfere with active motifs complicates their utility (28). For mixtures of DNA vaccines with CpG oligonucleotides, it appears that the oligonucleotides interfere with transfection by plasmid DNA, as reporter gene expression is reduced (29). Delivery of DNA directly to the cytoplasm of cells in situ, through the use of electroporation, can abrogate the inhibitory effects of CpG oligonucleotides (M. J. Selby, et al., unpublished observations), indicating that the competition between plasmid DNA and CpG oligonucleotides is manifest at the level of DNA uptake by cells. Hence, appropriate formulation and/or delivery of DNA plus CpG oligonucleotides will be required to take advantage of the immunostimulatory effects of CpG.

DNA Vaccine Delivery

A third approach is to facilitate DNA delivery into cells. Potential barriers to transfection include:

1. lack of widespread distribution of DNA within the inoculated tissue;
2. rapid degradation of unprotected DNA;
3. inefficient uptake of DNA by cells (either directly through the plasma membrane or by endocytosis);
4. degradation of DNA within the endosome/lysosome; and
5. inefficient uptake of DNA by the nucleus, particularly in nondividing cells where the nuclear membrane remains intact.

These limitations may explain why only a small fraction of muscle cells are detectably transfected (3) and only ~1 in 10^7 molecules of injected plasmid DNA can be recovered from a mouse muscle after 7 days (30). Although APCs also can be transfected (31–33), the efficiency appears to be even less than in muscle cells. Studies with fluorescently tagged plasmid DNA revealed that much of the inoculated DNA was phagocytosed by macrophages within the muscle, with much less found in muscle cells, and little or none detected within the nuclei of any cells (M. Dupuis, et al., accepted for publication). Therefore, means to facilitate egress of DNA out of endosomes or bypassing this pathway altogether should be profitable. Following are descriptions of two technologies that may be able to accomplish this.

First, electroporation in vivo has been used to facilitate DNA delivery directly into cells, resulting in increased expression levels (34) and DNA vaccine potency (35). The enhanced immunogenicity was seen as higher levels of both antibodies and T-cell responses in mice. With regard to the latter, CD8+ T-cell responses were

quantified by measuring IFN-γ production after brief restimulation with a MHC class I-restricted peptide epitope. Electroporation also was effective at increasing antibody responses in guinea pigs and rabbits. The means by which this technique enhances DNA vaccine potency is not yet known. But, electroporation long has been used to transfect cells in vitro and is thought to cause transient disruption of the plasma membrane, thereby, allowing DNA to enter cells directly and bypass the less efficient route of endocytosis. Because plasmid DNA is highly charged, the application of an electric current may also facilitate movement of DNA within the tissue in a process termed iontophoresis, which has been used for transdermal delivery of small molecules. Finally, it is possible that the electric current causes an inflammatory response that attracts APCs to the site of DNA injection, thereby, acting as an "adjuvant." Based on our preliminary data, electroporation does not appear to enhance transfection of APCs; rather, expression is detected in many more muscle cells than without electroporation (M. Dupuis, et al., accepted for publication). These results suggest that increased antigen production by non-APCs yields stronger immune responses. They are consistent with previous observations that synthesis of antigen by non-APCs is sufficient to prime immune responses, including antibody and T-cell responses (36–40).

Second, targeting of APCs for transfection by DNA vaccines is a logical approach to increasing DNA vaccine potency, since these cells are potent inducers of immune responses against antigens expressed by DNA vaccines (33,39,41,42). One possible method to increase delivery of DNA to APCs in vivo is to use a particulate formulation of DNA to take advantage of the high phagocytic capacity of such cells, particularly immature dendritic cells. In support of this hypothesis, i.m. injection of poly(lactide-co-glycolide) (PLG) microspheres ~1 μM in diameter with surface-adsorbed DNA induced substantially higher levels of immune responses than naked DNA (43). Based on a dose-response titration in mice of naked DNA versus PLG/DNA, antibody and CD8+ T-cell responses were increased by 1000- and 100-fold, respectively. PLG/DNA also was found to substantially enhance antigen-specific antibodies in guinea pigs (O'Hagan, et al., unpublished observations). The mode of action of these particles has not yet been fully elucidated. However, preliminary results indicate that these particles do not substantially increase expression in muscle in vivo (43), but can transfect dendritic cells in vitro, resulting in processing and presentation of antigen to T cells (K. Denis-Mize, et al., submitted for publication). Therefore, targeting of DNA vaccines to both APCs and non-APCs may have beneficial effects, although probably through different mechanisms, such as expression of antigen within APCs versus cross-priming within non-APCs.

The feasibility of the two approaches described above as DNA vaccine delivery technologies remains to be determined, but the enhancing effects on DNA vaccine potency observed in animal models indicates that DNA delivery systems will be an important component of second-generation DNA vaccines.

Plasmid and Particle Alphavirus Replicon Vaccines

Alphaviruses have several properties that make them desirable as gene delivery vectors for both vaccine and gene therapy applications, including:

1. transient, high-level antigen expression;
2. broad tissue host range;
3. ability to infect both dividing and non-dividing cells;
4. induction of host immuno-stimulatory responses;
5. ability to infect antigen-presenting cells; and
6. both plasmid DNA-based and vector particle-based delivery formats.

Alphavirus vectors are "suicide vectors," since expression in infected host cells is always transient, through induction of apoptosis. The vector particles are propagation-incompetent, and infection of host cells does not proceed beyond a single round. The transient nature of alphavirus vectors may, in fact, provide an important safety advantage for this system. They avoid the theoretical potential of risks from immune tolerance related to persistent expression of antigen in vaccine applications, or transformation from prolonged expression of growth factors in gene therapy applications.

Although the alphavirus genus is rather large, comprising more than 25 species (44–46),

principally only three are being developed as vectors: Sindbis virus (SIN), Semliki Forest virus (SFV), and Venezuelan equine encephalitis virus (VEE). There now exists a large body of literature demonstrating the induction of robust and broad antigen-specific immune responses in rodents and non-human primates immunized with alphavirus-derived plasmid or recombinant particle vectors (45).

Alphaviruses are enveloped with an icosahedral shell containing a single-stranded, positive-sense RNA genome that is approximately 12 kb, capped, and polyadenylated. Gene expression from the alphavirus genome is segregated into two regions. The enzymatic nonstructural proteins (nsP1-nsP4), known collectively as the viral "replicase," are synthesized from the 5′ two-thirds of genomic-length RNA and catalyze the synthesis of progeny plus-stranded genomes through a negative-stranded RNA intermediate. The negative-strand RNA copy of the genome also serves as a template for the synthesis of a subgenomic mRNA, whose synthesis is initiated from a highly active internal mRNA promoter (known as the "26S mRNA" or "subgenomic" promoter), which is functional only in the negative-sense RNA. The subgenomic mRNA, which corresponds to the 3′ one-third of the genome and encodes the alphavirus structural proteins (sPs), may be produced at a 10-fold molar excess relative to genomic RNA in infected cells. The complete replicative cycle of alphaviruses occurs in the cytoplasm of infected cells (46).

Alphavirus vectors are essentially substitution vectors, in which the viral sP genes are replaced with an antigen-encoding gene. These vectors are so-called "replicons," because they retain the replicase genes from the parent virus encoding and, thus, can direct their self-amplification in the infected cell, resulting in high-level antigen expression. Both plasmid DNA and recombinant vector particle delivery formats for alphavirus replicons have been developed (47–53). Each system has its inherent advantages and is discussed below.

Alphavirus Plasmid DNA Replicons

While alphavirus replicons—as RNA or as infectious particles—have been used widely as expression vectors in cultured cells, as well as for vaccines in animal models of infectious disease, methods involving transcription in vitro of vector cDNA followed by transfection of RNAs to produce vector particles are inefficient. In addition, bacteriophage RNA polymerases are error-prone, increasing the possibility of mutations within the vector and heterologous gene. More recently, efficient methods for expressing alphaviral genomes from RNA polymerase II expression cassettes have been developed. This advance has resulted in the development of two alphavirus replicon delivery systems. The first system is a layered plasmid DNA-based vector (47,49). Unlike conventional expression vectors, the gene of interest is not translated from the primary transcript (first layer) synthesized in the nucleus. Rather, the replicase expressed from the transported vector RNA subsequently programs the synthesis of high levels of the subgenomic RNA (second layer) encoding the antigen, via a negative-stranded intermediate. The second system is based on recombinant vector particles and is described in more detail in a later section.

In two separate investigations, plasmid DNA-based alphavirus replicons have been shown to be significantly more efficacious in protecting vaccinated mice against lethal virus challenge (HSV-1 or influenza) than conventional plasmid DNA expression vectors (48,54,55). In both studies, the immune correlates of protection against lethal virus challenge included humoral and cellular responses. The CD4+ immune response in the alphavirus plasmid-immunized mice was primarily of the T_H1 type, as demonstrated by a high immunoglobulin (Ig)G2a/IgG1 ratio. For SIN-based DNA plasmid, CTL precursors were induced by replicons expressing herpes simplex virus (HSV) glycoprotein B at DNA dosage levels 1000-fold lower than conventional plasmid. More recently, these earlier results were extended across a range of intramuscular doses by comparing Sindbis virus replicon (pSIN) and conventional plasmid DNA vectors expressing human immunodeficiency virus (HIV) gp160. The results of these studies indicated that the alphavirus replicon plasmid was much more effective at inducing HIV gp160-specific CTL precursors than the conventional DNA plasmid.

The mechanism(s) responsible for the enhanced potency of plasmid DNA alphavirus replicons, compared with conventional plasmid expression vectors has not been defined. Despite the amplification of messenger RNA by the replicase, it is not clear whether there is a

substantial difference in antigen expression level between these two vectors. Although replicon-based transgene expression in cultured cells may reach 20% of the total cell protein (51), it is not known whether this level translates to in vivo, where interferon or other innate immune responses may limit the level of expression. Another possibility for the enhanced efficacy of the plasmid DNA replicon may be related to its expression strategy. Double-stranded RNA (dsRNA) is produced as a result of replicon amplification and may enhance the immune response through mechanisms of increased MHC class I-associated antigen presentation, and/or activation of dendritic cells (56). Additional mechanisms may include adjuvanting by the replicon replicase, or the induction of apoptosis in replicon-containing cells and cross-priming of antigen presenting cells (57).

Alphavirus Particle Replicons

Although alphavirus replicon particles were shown to be efficacious in animal models of infectious disease, economical means for their production at large scale — an important requirement for commercial application — were not available. Recently, stable alphavirus replicon packaging cell lines (PCL) were developed that can be used to produce recombinant alphavirus replicon particles (52). The PCL were generated by stably transforming mammalian cells with two helper expression cassettes separately encoding the SIN capsid and glycoprotein structural protein genes. Translation of structural proteins (sPs) was induced in the PCL only after synthesis of the helper-encoded subgenomic mRNA, catalyzed by the replicon-encoded replicase, which was introduced into the cell line via transfection with plasmid DNA replicon or infection with replicon particles. Production of replicon particles using PCL is a two-step process. In the first step, a seed stock of replicon particles is produced by transfecting the PCL with a plasmid DNA-based replicon. In the second step, a large stock of replicon particles is produced by infecting a fresh culture of PCL with the initial seed stock. In this process, particles produced after infection of the PCL with the seed stock, in turn, infect neighboring packaging cells and initiate another round of vector particle production. Amplification continues until all of the cells in the PCL culture are infected and producing progeny particles. Vector particles can be harvested from the PCL infected with seed stock at titers up to 10^8 infectious units (IU)/ml. Importantly, the replicon particle stocks produced by this method are, themselves, incapable of replication and are free from detectable contaminating replication-competent virus. Thus, methods have been developed to produce replicon particles that are not only amenable to large-scale manufacture and of a higher quality, due to RNA polymerase II-based expression. However, they also are anticipated to be much more cost-effective than methods based on in vitro transcription.

Alphavirus particle replicons are expected to be more efficient for in vivo production of encoded protein than plasmid DNA replicons, since viral infection is inherently more efficient than DNA transfection in vivo. Indeed, induction of influenza (flu) hemagglutinin (HA)-specific antibodies was demonstrated in mice vaccinated with as little as 100 SFV replicon particles (58). In general, immunization of diverse strains of mice with $1 \times 10^{4-7}$ SIN, SFV, or VEE replicon particles induces both humoral and cellular responses, and, in some cases, mucosal responses (59). Protection in vaccinated mice against lethal challenge with the infectious agent corresponding to the replicon-expressed antigen has been shown with flu (51,60) and herpes simplex virus (J. M. Polo, et al., unpublished observations), and in guinea pigs vaccinated with (GP)-expressing VEE replicon particles against the particularly virulent filovirus, Marburg (MBGV) (61). Success in smaller animals has led to testing of efficacy in vaccinated primates. The results of these early primate studies are promising as well. The MBGV investigation was extended to Cynomolgus monkeys (61). In both simian immunodeficiency virus (SIV) and simian-human hybrid immunodeficiency virus (SHIV) primate challenge models of HIV infection, immunization studies have been promising, although not to the extent of the MBGV investigation. Monkeys have been vaccinated with SFV or VEE replicon particles expressing either envelope or matrix/capsid from HIV or SIV and subsequently challenged with the corresponding virus, SIV or SHIV. The general observation is induction of both humoral and cellular antigen-specific immune responses and a reduction in the viral load, compared with unvaccinated controls (62,63). Rhesus macaques immunized multiply with VEE replicon particles were protected against disease for at least

16 months post-challenge with a pathogenic SIV swarm, and had 100-fold lower viral load levels than nonim-munized control animals (64). Because viral load is predictive of time of progression to acquired immunedeficiency syndrome (AIDS) in HIV-infected individuals, these results are indeed encouraging.

Since effective vaccines against a number of infectious diseases that are worldwide health problems, including HIV and hepatitis C virus (HCV), have to date remained elusive, multiple strategies and vector modalities of gene-based vaccines are being tested. One reason for the recent increased attention for alphavirus replicons is the demonstration that both VEE- and SIN-based particles directly target antigen-presenting cells. Following subcutaneous inoculation, VEE replicon particles infect resident Langerhans cells, which migrate subsequently to the draining lymph node following activation (65). One of the two alphavirus envelope glycoproteins comprising the spike structure that protrudes from the replicon particle envelope, E2, is a primary determinant of this specificity, as shown by a loss of targeting to Langerhans cells with a particular VEE mutant (65). In another approach, SIN variants that exhibited high-level productive growth in immature human dendritic cells (DC) were derived. Similar to VEE, the genetic determinant for this phenotype mapped to a single amino acid codon (although distinct from VEE) in the E2 gene (J. P. Gandner, et al., submitted for publication). The ability of the DC-tropic SIN variants to infect dendritic cells was characterized using replicon particles encoding the green fluorescent protein (GFP) reporter protein. In the hematopoietic lineage, only immature cultured DC could be infected, with up to 30–50% of cells transduced routinely by one variant, at relatively low multiplicities of infection. Mature DC, as well as T cells, B cells, monocytes, and natural killer (NK) cells, were refractory to infection, even at high multiplicities of infection (>100). Costimulatory (CD80, CD86) and MHC molecules were up-regulated on transduced immature DCs, emphasizing the natural adjuvant activity of the SIN vector particles. Thus, SIN replicon particles were developed that could infect cultured human immature dendritic cells with high efficiency, as well as infect resident Langerhans cells at the site of injection in mice. This resulted in trafficking to the draining lymph node where antigen could be presented. In the same study, SFV-derived replicon particles were unable to infect cultured human immature dendritic cells. It remains to be determined whether DC-targeted alphavirus replicon particles will have an increased potency for stimulating an immune response, compared with cognate particles lacking this cell tropism. This remains an open question, since in the context of at least infectious lymphocytic choriomeningitis virus, productive virus infection of DC results in systemic immunosuppression (66).

Conclusions

Both alphavirus replicon plasmids and particles have been shown to be efficacious in preclinical animal models of infectious disease. Although the replicon particles may indeed prove to be more potent, the replicon plasmids have a safety advantage, due to the avoidance of issues related to replication-competent virus (RCV). In addition to an antigen, both the replicon particles and replicon plasmids express the vector-specific replicase proteins. However, this would not seem to present a safety issue because the replicase proteins are not associated with any pathogenicity, unless in the context of infectious virus (67). Furthermore, any present vector-specific immune responses do not appear to preclude boosting existing antigen-specific immune responses with replicons encoding the same antigen, or the extent of a primary immune response to a novel antigen (51). Similar to any virus-based gene delivery system that has been tested in human clinical trials, it will be important to develop highly sensitive assays to ensure that replicon particle preparations are free from contaminating RCV. The risk of adverse events as a result of undetected RCV contaminating clinical preparations of alphavirus replicon particles must also be considered. The degree of this risk may be somewhat related to the pathogenicity of the parent alphavirus. In the case of VEE, while infection with the parent alphavirus can cause encephalitis and even death, multiple attenuating mutations have been engineered into the virus to strip it of its virulence, if a vaccine were injected with RCV. On the other hand, the extent of undetected RCV contamination, if any, would likely be orders of magnitude less than the level incurred from an alphavirus-infected mosquito during a blood meal. Even at this dramatically higher dose of wild-type virus, infection with alphaviruses is usually inapparent or self-limiting.

Is one replicon derived from a particular

alphavirus superior? The answer to this question is presently unknown. For practical, if not commercial, reasons it will be important to compare these (and possibly other) alphaviruses directly with each other for efficacy in pre-clinical as well as clinical studies. Decisions of which alphavirus replicon to develop clinically will be related in part to safety, potency, and ability to manufacture. Taken together, the combination of alphaviral vector formats that are amenable to commercial-scale manufacture, together with multiple reports of efficacy in both rodent and primate models of infectious disease, indicates that the future of alphavirus replicons as an effective vaccine modality for humans is indeed a bright one.

Summary

Several approaches for increasing the potency of gene-based vaccines were presented. These include means of increasing DNA delivery to cells through physical methods (e.g., electroporation) and formulation [e.g., (PLG) particles]. In addition, the utility of using RNA replicons such as alphaviruses has been shown for both particle- and DNA-based systems. Because of the effectiveness of prime-boost strategies involving DNA followed by live vectors, such as vaccinia (68) and avipox (69), it will be particularly interesting to evaluate the potential synergy between DNA and alphavirus replicons. These technologies, separately or together, show promise for effectiveness in humans.

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