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The promise of nucleic acid vaccines

NP Restifo, **H Ying**, **L Hwang**, and **WW Leitner**
Surgery Branch, National Cancer Institute, Bethesda, MD, USA

Abstract

Establishing the effective use of 'naked' nucleic acids as vaccines would undoubtedly be one of the most important advances in the history of vaccinology. While nucleic acids show much promise for use as vaccine vectors in experimental animals, not a single naked nucleic acid vector has been approved for use in humans. Indeed, data from human clinical trials is scant: nucleic acid vaccines have not been clearly demonstrated to have any convincing efficacy in the prevention or treatment of infectious disease or cancer. Here we illustrate possible mechanisms underlying effective nucleic acid vaccination. We focus on progress that has been made in the improvement of their function. Additionally, we identify promising new strategies and try to forecast future developments that could lead to the real success of nucleic acid vaccines in the prevention and treatment of human disease.

Keywords

DNA vaccines; self-replicating vectors; dendritic cells; RNA vaccines; danger signals; apoptosis

How do nucleic acid vaccines work?

It has been a decade since workers found that injection of 'naked' plasmid DNA, that is DNA without any associated lipid, protein or carbohydrate, could elicit an immune response.[1,2] This unexpectedly successful new method has spawned a whole new scientific field and led some to declare the new method one of the most important discoveries in the history of vaccinology. While the earliest studies were done using DNA, some subsequent studies have explored the use of RNA vaccines. We therefore refer to them collectively as nucleic acid vaccines.

There are several reasons why naked nucleic acids are attractive candidate vectors for the development of vaccines for infectious diseases and cancer. They are relatively simple to generate and safe to administer. In contrast to vaccines that employ recombinant bacteria or viruses, genetic vaccines consist only of DNA or RNA, which is taken up and translated into protein by host cells. The simplicity of their composition has immunological advantages. Because they are not associated with a viral coat, naked nucleic acids are not generally subject to neutralizing antibody reactions that can hamper the clinical efficacy of vaccines based on recombinant viruses. Further, the lack of a viral coat eliminates the possibility that immune responses to the coat will be immunodominant over immune responses to the desired transgene product.

Unfortunately, immunization with naked nucleic acid is relatively inefficient and virus vectors generally induce far greater immune responses than DNA vaccines. The coating of the nucleic acid cores of viruses both protects the viral genome from degradation and facilitates entry into the host cell. Thus, a virus might be viewed as making an evolutionary trade off: although the acquisition of a viral coat results in a loss of stealthiness, this coat vastly increases the virus's replicative efficiency.

Correspondence: N Restifo, Surgery Branch, NCI, Bldg 10, Room 2B42, 10 Center Dr, Bethesda, MD 20892, USA.

How then do nucleic acid vaccines enter host cells? Unlike viruses, naked nucleic acids lack the aid of proteins, and in some cases lipids and sugars, that are so critical to viral infection. One answer is that cells spontaneously take up nucleic acids. For example, myocytes have been shown to take up DNA, which can then be transcribed and translated to produce protein.[3] Vaccinologists have tried to increase this uptake by the addition of cationic lipids capable of binding to DNA and facilitating the transposition of the DNA/lipid complex across the cell and possibly nuclear membranes. Another solution has been to physically blast the DNA into the cell using a 'gene-gun'. Plasmid DNA is precipitated on to an inert particle (such as a gold bead) and forced into the cells with a helium blast. The most commonly used method of delivery for nucleic acid vaccines, however, remains needle injection into muscle or skin. Alternatively, the mucosal surfaces of the respiratory, digestive and reproductive systems have been explored as potential sites of immunization, due to their importance as barriers to (and portals of) the transmission of infectious diseases.

How do nucleic acid vaccines elicit immune responses?

On the simplest level, nucleic acid vaccines employ the host's transcriptional and translational machinery to produce the desired gene product. This polypeptide product can then be recognised by components of the immune system. But how does recognition and activation of immune cells occur? Early work focussed on the uptake of plasmid DNA by myocytes. Although myocytes can present antigen to immune cells, myocytes are not the primary activators of immune cells. Instead, an immune response is primarily launched by specialized bone marrow (BM)-derived antigen presenting cells (APC) called dendritic cells (DC). The crucial role of bone marrow-derived DC has been established primarily by using BM-reconstituted chimeras.[4-6] The central role of DC is not unique to nucleic acid vaccines. For example, in the case of recombinant poxviral immunogens, the strongest immune response is elicited by constructs with promoters that function best in DC.[7] Likewise, the function of DNA immunogens should be optimized in DC.[8]

How are antigens that are encoded by nucleic acid vaccines captured by APC? DC, the most powerful APC, acquire antigen by three primary routes. First, DC can be directly transfected with nucleic acid vaccines.[9] Second, DC take up soluble antigen from interstitial spaces that has been secreted or released by transfected cells. Third, and perhaps most interestingly, DC preferentially take up cells that have been injured or killed as a result of the vaccine or its function.

Uptake and presentation of antigen is only the first step in the functional activation of DC. Upon activation, DC will express and up-regulate the required surface adhesion molecules and chemokine receptors that enable them to migrate to lymphatic organs where they are most effective at activating immune responses.

While collateral damage caused by gene gun immunization can function as a 'danger signal' that effectively activates DC,[9] one question remains: why would the uptake of antigen from transfected somatic cells activate DC? Antigen produced by transfected cells without any 'danger signals' is expected to be non-immunogenic as is protein-antigen injected without adjuvants. We propose that cell death induced by the transfection of host cells is the signal for the activation of dendritic cells by providing the necessary 'danger signals',[10] though there remains some contention about whether this death be apoptotic or necrotic. Cells transfected *in vitro* with conventional DNA vaccines express widely varying amounts of antigen, presumably due to the number of plasmid copies that were picked up.[11] Only cells harboring high copy numbers of DNA vaccine will die as a consequence of the transfection (unpublished observation). This leads us to hypothesize that the unsatisfactory efficacy of conventional DNA vaccines delivered by needle injection might be due to its relative immunological 'blandness'.

Improved function might be obtained by increasing the associated 'danger' emanating from the transfected cells.

We have demonstrated that cells harboring replicon-based naked DNA or RNA vaccines eventually die as a consequence of the 'self-replicating' activity of the vaccines, and such dead cells were taken up by APC *in vitro*.[12] In addition, we have also found that uptake of the conventional DNA vaccines in a specific population of cells also results in cell death (unpublished observation). We propose that the cell death induced at the site of vaccination with DNA enables the uptake by, and subsequent activation of, local or migrating DC. These DC can migrate to lymph nodes where they can process DNA vaccine-derived antigen in the context of both MHC class I and class II molecules for presentation to $CD8^+$ and $CD4^+$ T cells.

How can nucleic acid vaccines be improved?

One great advantage in the use of recombinant and synthetic immunogens, including nucleic acid vaccines, is the control that the vaccinologist has over how the antigen is presented to the immune system for recognition. Starting with a cDNA encoding an antigen in its native form, sequences can be added or subtracted to improve the immunogenicity of an antigen encoded by a nucleic acid vaccine. For example, the intracellular or trans-membrane domains can be removed to decrease toxicity or improve solubility.[13] Alternatively, sequences can be added to target antigens to the MHC class-I or class-II processing pathways.[14,15] Further, epitopes can be modified to increase their abilities to bind to MHC molecules, thereby increasing their immunogenecity.[16] Antigens can be truncated creating minigenes that only encode the immunodominant epitopes,[17] or they can be buried within unrelated, but highly immunogenic core sequences. This may be especially useful in cases where full-length proteins are not suitable as vaccine candidates, because they are toxic for the host or immunosuppressive.

In addition to modifying the form of the antigen that is encoded within the nucleic acid vaccine, the vaccinologist can also tinker with the micro-environment in which the antigen is presented to the immune system.[18] A number of immunomodulatory molecules can be used to augment immunization. One group called cytokines is comprised of soluble molecules that generally act locally on immune cells with a limited half-life in circulation. Cytokines can enhance vaccine function through a number of mechanisms.[19-21] For example, interleukin-2 (IL-2) can cause the activation and proliferation of T lymphocytes. Other cytokines can 'steer' the immune response by preferentially augmenting the function of some immune cells over others. In the case of IL-12, the Th1 subset of $CD4^+$ T cells is enhanced while the Th2 subset is diminished. Co-delivery of cytokines or cytokine genes can also be used to recruit and mature dendritic cells. The most striking example of this type of cytokine may be granulocyte–monocyte colony-stimulating factor (GM-CSF).[21,22]

Co-stimulatory molecules are also potentially powerful adjuvants for nucleic acid vaccination. Signaling through the interaction of TCR and peptide/MHC is essential but insufficient to activate a T cell. In order to become fully activated, T cells require costimulation. T cells receive a large number of second signals upon their interaction with an APC. These signals are mediated, in part, through the interactions of intracellular adhesion molecules (ICAMS), lymphocyte function associated antigens (LFAs) and the B7 molecules (called B7.1 or CD80 and B7.2 or CD86). The mere inclusion of a costimulatory molecule in a nucleic acid vaccine may not ensure immune activation. For example, the B7 molecules can either activate T cell responses if they engage the CD28 ligand, or inhibit T cell response, if they bind to CTLA4.[23]

The adjuvant potential of another group of immune-signaling molecules, called chemokines, has not yet sufficiently been explored for nucleic acid vaccines. Chemokines are cytokines with chemoattractant capacities. They can induce the activation and directional migration of a

variety of immune cells. Therefore, they can be used to condition an injection site or can be co-delivered with nucleic acid vaccines to preferentially attract immune cells with desired functions, such as DC or particular subsets of lymphocytes.[24]

Finally, much has be written about the adjuvant effects of immunostimulatory sequences (ISS).[25,26] These nonmethylated, palindromic DNA sequences containing CpG-oligodinucleotides can activate B cells, T cells, NK cells and DC *in vitro* and *in vivo*. Increased numbers of ISS can either be incorporated into the backbone of nucleic acid immunogens, or co-administered with plasmid-DNA in the form of oligonucleotides.[27,28]

The use of alphaviral replicons can increase the efficacy of nucleic acid vaccines

A most recent improvement upon plasmid nucleic acid vectors was the incorporation of alphavirus replicons. 'Self-replicating' or replicon-based genetic vaccines were designed to overcome the poor efficacy of some current DNA-based and RNA-based genetic vaccines. This new generation of genetic vaccines takes advantage of the replication machinery used by members of the Alphavirus genus, which includes Sindbis virus, Semliki Forest virus (SFV), and Venezuelan equine encephalitis (VEE) virus. The alphaviral genome consists of a single copy of positive-stranded RNA. This RNA encodes structural genes, as well as a single non-structural gene encoding an enzyme that is capable of copying RNA. This replicase gene is transcribed as a single autoproteolytic polyprotein, which cleaves itself into four subunits (nsP1–4). Upon infecting a cell, the viral RNA first translates the replicase complex, which in turn synthesizes a genomic negative-strand (anti-sense RNA) that is used as a template for the synthesis of the genomic positive-stand RNA. The negative-stranded copy of the genome also serves as a template for a subgenomic RNA encoding the structural viral proteins. By replacing the genes for structural proteins of the virus with an antigen of interest, a 'self-replicating' RNA vaccine can be generated.

To increase the stability of the construct and to facilitate the production and handling of the vaccine, the self-replicating RNA can be encoded by a DNA plasmid where a CMV promoter 'kick-starts' the production of the self-replicating RNA. The alphavirus replicase functions in a broad range of host cells (mammalian, avian, reptilian, amphibian and insect cells). Replication takes place in the cytoplasm of the host cell and, therefore, is independent of the host's replication system making replicase-based nucleic acid vaccines a very efficient and attractive delivery vehicle.

We have recently demonstrated that both an RNA vaccine as well as different plasmid DNA replicons encoding a model tumor-associated antigen (TAA) under the control of alphaviral RNA replicase are effective in the treatment of an experimental tumour.[11,12] In animal models of infectious disease, these plasmid DNA replicons are substantially more efficient at stimulating antigen-specific immune responses, particularly cellular responses, as compared with conventional plasmid DNA expression vectors. Alphavirus replicons, in the form of RNA, DNA or infectious particles can be potent inducers of broad immune responses in both rodents and primates.

Mechanisms underlying the increased efficacy of self-replicating nucleic acid vaccines

Replicase-based nucleic acid vaccines may employ qualitatively different mechanisms for immune activation as compared with 'conventional' DNA vaccines. The initial rationale for putting antigen-coding genes under the control of the alphaviral RNA replicase was to enhance antigen expression. The fine regulation of the host transcriptional machinery can impede RNA

production by conventional plasmid DNA, but host transcription is not required using RNA replicon vaccines. Further, the activity of the RNA replicase amplifies the level of RNA present in the cells. Unexpectedly however, the level of antigen expression of replicase-based constructs *in vitro* is not necessarily higher than that obtained with conventional DNA vectors.[12,29] The discordance between antigen expression level and the increase in immunogenicity suggests that other mechanisms are responsible for enhanced immunogenicity. A fundamental difference between replicase-based DNA vaccines and conventional DNA vaccines is the virus-like RNA-replication inside transfected host cells, which could trigger a series of 'danger signals' by mimicking a viral infection of the transfected cell. A key event in this process might be the production of abundant double stranded RNA (dsRNA) which are the requisite intermediates of RNA replication.

dsRNA that is produced as a result of alphaviral infection is known to trigger two major antiviral mechanisms of the host cell: the protein kinase-RNA activated (PKR) and 2′–5′-oligoadenylate (2–5A) synthetase pathways. Activation of the 2–5A system contributes to the antiviral effect of the interferons through the synthesis of 2–5A and its subsequent activation of RNase L, which degrades both viral and cellular RNA. Induction of the PKR antiviral pathway by dsRNA up-regulates host cell interferon production and also triggers inhibition of translation. The activation of both of these pathways predisposes the cell to death by apoptosis.[30-32] Indeed, transfection of cells with self-replicating RNA as well as with plasmid DNA replicon causes apoptotic death as does the infection with the complete alphavirus.[12,29] These dead, antigen-loaded cells could then be picked up by APC, which might be activated by 'danger' signals released in response to the transfection and the presence of viral replicase.

Although apoptosis of cells transfected with self-replicating nucleic acid vaccines may contribute to the observed immunogenicity, the rapid death of host cells due to these same death responses could also limit the efficacy of replicase-based nucleic acid vaccines. The incorporation of viral and cellular modulators of cell death, including vaccinia E3L, HIV-1 Tat protein or the cellular P85IPK, into replicase-based vaccines may enable us to disentangle the mechanisms of increased antigen production and increased apoptotic death. Elucidation of innate cellular responses, which are important in the induction of the immune responses by replicase-based nucleic acid vaccines may also make it possible to enhance their function further.

Clearly, nucleic acid vaccines have come a long way in the past decade. These vaccines offer a number of advantages: they are easy to generate and manipulate, they can be safely administered, and they can be used in multiple immunizations. Despite these advantages, advancements in nucleic acid vaccine development are still hindered by our limited understanding of the mechanisms of their activity. Advances in the formerly disparate areas of molecular biology, basic immunology and fundamental virology will all undoubtedly contribute to our burgeoning knowledge of how to design better nucleic acid vaccines in the new millennium.

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