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COMMENTARY

Nucleic acid-based vaccines targeting respiratory syncytial virus: Delivering the goods

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ABSTRACT

Respiratory syncytial virus (RSV) is a massive medical burden on a global scale. Infants, children and the elderly represent the vulnerable populations. Currently there is no approved vaccine to protect against the disease. Vaccine development has been hindered by several factors including vaccine enhanced disease (VED) associated with formalin-inactivated RSV vaccines, inability of target populations to raise protective immune responses after vaccination or natural viral infection, and a lack of consensus concerning the most appropriate virus-associated target antigen. However, with recent advances in the molecular understanding of the virus, and design of highly characterized vaccines with enhanced immunogenicity there is new belief a RSV vaccine is possible. One promising approach is nucleic acid-based vaccinology. Both DNA and mRNA RSV vaccines are showing promising results in clinically relevant animal models, supporting their transition into humans. Here we will discuss this strategy to target RSV, and the ongoing studies to advance the nucleic acid vaccine platform as a viable option to protect vulnerable populations from this important disease.

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Introduction

Each year 33.8 million Infants and children become inflicted with lower respiratory disease after RSV infection, leading to 3.4 million hospitalizations with severe disease complications.[1,2] Furthermore, an inability of natural infection to mount robust long-lived immunity can leave immunosenescence populations, such as the elderly, vulnerable to respiratory complications after exposure. Currently the treatment option outside of high resource countries is limited to supportive care, immunoprophylaxis with the neutralizing antibody palivizumab being cost-prohibitive. Though considered a high medical need there is no approved RSV vaccine available. However, there is heavy investment into vaccine development. The high level of vaccine development activity is revealed on the PATH (www.PATH.org) snapshot of the current landscape. It reveals 40 candidates in preclinical development, and 14 at various clinical stages. Even though the most advanced candidate – a RSV-F targeting VLP-based vaccine, recently reported disappointing topline efficacy results in a phase 3 study in elderly adults (9.15.2016 Novavax press release) there are many other promising vaccine candidates under development on a variety of platforms including, live-attenuated/chimeric, whole-inactivated, particle-based, subunit and gene-based vector. This commentary article will focus on nucleic acid-based vaccines targeting RSV.

In vivo delivery of nucleic acid-based vaccines

At the beginning of the 1990's Wolf and colleagues reported *in vivo* protein expression after intramuscular injection of plasmid DNA or mRNA into mice.[3] It was this discovery that marked the beginning of the use of nucleic acids encoding antigens as a form of vaccination. While the instability of mRNA limited its use, plasmid DNA offered a very promising new vaccine platform. pDNA was stable, it could be produced both rapidly and in bulk, the transgene could be designed to encode antigen of choice, and the pDNA-vectored antigen could be delivered multiple times to boost immunity (pDNA itself is not immunogenic, and the issues associated with anti-vector immunity can be avoided). Studies in small animals revealed an attractive profile of both immunogenicity and safety.[4,5] However, initial studies in larger animals and humans disappointed, lower levels of immunogenicity were observed than those predicted from small animal models.[6] One major reason cited for this inconsistency was inefficiency of *in vivo* gene delivery. In response to this investigators in the field began developing both physical (electroporation (EP), ultrasound, gene gun) and chemical (lipids, polymers) *in vivo* delivery strategies to enhance the passage of pDNA into the host cell. [7-12] In recent years electroporation has become the go to delivery aide for nonviral gene delivery. Studies have consistently shown 100–1000 fold enhanced *in vivo* gene expression upon the employment of electroporation to protocols delivering naked pDNA.[8] EP gene transfer operates by inducing transient perturbations in the cell membrane and an electrical gradient which promotes the passage pDNA into the cell. Importantly, employment of EP into DNA vaccine protocols has significantly enhanced immune responses in both small and large animals, permitting protection against pathogen in challenge models. [13-17] Multiple DNA vaccine trials are successfully employing this technology to elicit robust host immune responses, and clinical efficacy of this platform has now been reported.[18,19]

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RSV nucleic acid-based vaccines 1.0

With an impressive safety profile, ability to stimulate humoral and cellular immune responses, and the capability of the investigator to design the vaccine to express only the desired antigen target, DNA vaccines may be an ideal platform to tackle RSV. Additionally, DNA vaccines exhibit the capacity to drive potent immune responses skewed towards Th1, which is a desirable trait considering the lung inflammation associated with the VED responses after FI-RSV vaccination have been attributed to dysregulated Th2 responses.[20]

In 1998 Li and colleagues designed a DNA vaccine to target the RSV fusion (F) glycoprotein and demonstrated intramuscular immunization elicited strong Th1 responses, neutralizing antibodies and cytotoxic T cells in mice, and also achieved protection from disease challenge.[21] Many RSV vaccines have been designed to target the F protein, which is a confirmed target for neutralizing antibody and CTL responses in human. [22-24] The FDA-approved immunoprophylactic monoclonal Palivizumab targets antigen site 2 on the RSV F fusion protein.[23] Another vaccine target is the G glycoprotein, which is less well conserved than the F glycoprotein across the RSV subgroups.[25] While initial studies with non-DNA vaccine platforms suggested RSV G antigen responses to be polarized towards Th2,[26,27] and thus promoting atypical lung inflammation after live RSV exposure, in contrast vaccine studies using DNA revealed a more balanced Th1/Th2 in the cotton rat model.[28] Cotton rats are considered the gold standard small animal model to study RSV infection, being susceptible to non-adapted RSV and displaying many features of human lung pathology.[29]

RSV nucleic acid-based vaccines 2.0

Almost 20 years has passed since the first description of RSV nucleic acid-based vaccines, but no candidate is in the clinic. For the reasons discussed above concerning difficulties involved in scaling-up and retaining immunogenicity form small animals to large animals and humans, has hampered the RSV DNA vaccine field's progress. However, since the first wave of DNA RSV vaccines several important advances have occurred in the field. These include codon optimization,[30] further understanding and rational design to specific regions or protein structure (pre- or post-fusion F glycoprotein) of the RSV antigen, [31-33] and delivery of the vaccine.[8,34] For DNA-based vaccines it is the improved delivery strategies which have made this platform relevant again. Electroporation has been employed to deliver DNA-based RSV vaccines to achieve robust immune responses in both small and large animals. [35-38] In an intramuscular (IM) delivery protocol Grunwald *et al.* demonstrated enhanced levels of immunogenicity of a RSV-F DNA vaccine with the addition of IM EP compared to conventional IM delivery in rhesus macaques.[35] The group also described increased immunogenicity upon delivery of the vaccine to the skin with EP. Our group has recently investigated both RSV-F and RSV-G DNA vaccines delivered with EP to the muscle or skin. Results revealed stronger T cell and neutralizing antibody responses after RSV-F compared to RSV-G DNA vaccination in experimental models including mice, Wistar rats, cotton rats and nonhuman primates[34] (and manuscript in preparation). Both RSV-F and RSV-G DNA vaccines delivered with EP protected from lower respiratory disease after RSV challenge in mice and cotton rats. While these studies demonstrate proof of concept, the method of EP and site of delivery must be appropriate to the disease indication. IM EP is an invasive procedure and as such is likely suited to certain vaccine targets in an adult population. With this in mind we have advanced a non-invasive delivery strategy with improved tolerability profiles. Our current focus is on surface EP (SEP) as a means to deliver a DNA RSV vaccine. This EP approach does not penetrate the live skin layers and operates at low voltages to produce only a shallow electrical field to target the pDNA vaccine to the epidermis. The epidermis is highly enriched in dendritic cells and is a site which permits dose sparing. We recently reported full protection against lower respiratory disease in the cotton rat RSV/A challenge model after a single low dose of a DNA based pRSV-F vaccine delivered at the skin using the SEP device.[34] We demonstrated the ability of this strategy to elicit robust immune responses. In contrast to the formalin-inactivated RSV (FI-RSV) vaccine, post mortem histological examination of the lung tissue revealed no enhanced lung inflammation upon virus challenge after DNA vaccination and thus no evidence of vaccine-enhanced disease, supporting vaccine safety. This dataset supports the advancement of a DNA vaccine candidate combined with an appropriate delivery platform to target vulnerable populations, including infants.

Arrival of the mRNA platform

In addition to the established pDNA platform, another branch of the nucleic acid vaccine family, RNA based vaccines, are beginning to show promise. [39-41] Although burdened by issues surrounding instability and manufacturing difficulties, many RNA vaccines researchers are making significant progress in overcoming these hurdles. Impressive preclinical and clinical immunogenicity data is now being reported on a variety of disease targets.[39,41,42] In 2013, Geall and colleagues reported immunogenicity and protection against disease challenge in cotton rats after 2 rounds of IM immunization with an RSV- naked self-amplifying RNA vaccine. Upon formulation of the RNA vaccine with lipid nanoparticles (LNPs), there was significantly enhanced immunogenicity and reduction in lung viral titers after viral challenge. Historically as was the case with DNA vaccines, naked delivery of mRNA appears to be suboptimal and data suggests that delivery optimization is required. LNPs have been shown successfully employed to enhance *in vivo* delivery of RNA vaccines.[43] Currently CureVac has a protamine-complexed mRNA RSV vaccine candidate in preclinical development.[41]

Conclusions

The field of nucleic acid-based vaccines has seen significant scientific and clinical progress since their inception in the early 1990's. Specifically, significant clinical efficacy data for EP-enhanced DNA vaccines has now been generated, and promising early development data for RNA vaccines looks encouraging. With improved and optimized delivery platforms, the historical hurdle of low vaccine immunogenicity has been

addressed, and ground breaking clinical efficacy is now being reported.[18,19] Though a nucleic acid-based RSV- vaccine has yet to reach the clinic, multiple promising candidates are in the development stages, both DNA[33] and mRNA-based.[40] Preclinical studies have revealed the desirable Th1 responses and strong protection in relevant animal models. With the advances in design and development of appropriate delivery platforms to immunize vulnerable populations we would expect clinical trials of these candidates to start soon.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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