# Virus *vs*. virus: adenovirus vectored vaccine to defeat respiratory syncytial virus

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*Provenance:* This is a Guest Editorial commissioned by Section Editor Dr. Mingzhu Gao (Department of Laboratory Medicine, Wuxi Second Hospital, Nanjing Medical University, Wuxi, China).

*Comment on:* Green CA, Scarselli E, Sande CJ, *et al*. Chimpanzee adenovirus- and MVA-vectored respiratory syncytial virus vaccine is safe and immunogenic in adults. Sci Transl Med 2015;7:300ra126.

Submitted Sep 29, 2016. Accepted for publication Oct 11, 2016.
doi: 10.21037/atm.2016.12.08
**View this article at:** http://dx.doi.org/10.21037/atm.2016.12.08

RSV is the single most important vaccine-preventable pediatric disease without a vaccine program. Fears of recurrence of the clinical experience with a formalin-inactivated RSV (FI-RSV) vaccine candidate in the 1960s, which led to enhanced respiratory disease (ERD) and death following natural infection (1), continue to cast a shadow on the development of RSV vaccines for use in infants.

RSV infection is one of the leading causes of hospitalization and health care visits in children <5 years of age and places a substantial strain on health-care infrastructures. Worldwide, RSV pneumonia is the second biggest cause of post-neonatal infant mortality, after malaria, causing an estimated 137,000 deaths/year (2).

Most children are infected with RSV at least once in the first 2 years of life. In one US cohort study, the cumulative rate of infection was 68% in the first 12 months and 97% before 24 months of age (3). Reinfection occurs frequently in early childhood, and recurs periodically throughout life. Severity of disease tends to diminish with subsequent reinfections until late in life, but in the frail elderly population the disease burden attributable to RSV is comparable to the burden attributable to influenza virus (4).

RSV is a pneumovirus in the Paramyxoviridae family of single-stranded, negative-sense RNA viruses. There are two major subtypes of RSV (RSV-A and RSV-B) that are distinguished by variation in the G-glycoprotein, which is one of the viral proteins embedded in the lipid envelope together with F (fusion) and SH (small hydrophobic). Most of the neutralizing activity in human serum is directed against the F glycoprotein (5), which is highly conserved among the RSV-A and RSV-B subtypes. Both subtypes circulate as seasonal epidemics in temperate climates, but in tropical climates can be detected throughout the year (6).

The natural history of RSV disease is the consequence of the virus tropism for the ciliated epithelia of small bronchioles and type I pneumocytes of the alveoli (7). The subsequent immune response results in the accumulation of mucus, sloughed epithelium and lymphoid aggregates that obstruct the bronchioles, which partially explains why infants—who have narrower and higher resistance small airways—are more prone to severe bronchiolitis (8). The mechanism by which the FI-RSV vaccine caused ERD and death, however, differed from the immune responses and pathology associated with natural infection, as the vaccine induced a higher titer of binding antibodies relative to the titer of functional neutralizing antibodies, resulting in immune complex deposition and complement activation (9). Additionally, RSV challenge studies in FI-RSV immunized mice showed that the vaccine induced the skewing of CD4+ T-cell immunity to a Th2 profile characteristic of allergic inflammation (10). The finding of abundant lung-infiltrated eosinophils in fatal cases of FI-RSV induced ERD might reconcile with the hypothesis that the vaccine induced a Th2 skewed immunological profile, as observed in mouse studies.

Immune correlates of protection from RSV infection and disease are not clearly established. There are evidences that a monoclonal neutralizing antibody specific for the

F protein can protect infants against severe RSV (11). However, levels of pre-existing serum neutralizing activity only marginally correlate with the protection from RSV infection in naturally infected elderly patients (12) or experimentally infected young adults (13). Therefore, it is likely that vaccines based only on the induction of neutralizing antibodies will have to induce very high and durable responses to be effective. The recent failure of a vaccine candidate based on F-protein recombinant nanoparticles to meet efficacy endpoints in a Phase III trial in 11,856 adults aged 60 and older (http://ir.novavax.com/phoenix.zhtml?c=71178&p=irol-news&nyo=0) seems to confirm this hypothesis.

On the other hand, human challenge studies have provided evidence that pre-existing intraepithelial (tissue-resident) CD8+ T-cells are negatively correlated with disease severity (14). Overall, the weight of evidence suggests that vaccine approaches that can induce CD8+ T-cell responses together with neutralizing antibodies in both antigen-naïve infants and in seropositive elderly would have value for virus clearance and, mainly in the case of pediatric vaccines, for producing IFN-γ to promote a Th1-biased T-cell response.

The primary goal of an RSV vaccine is to prevent severe lower respiratory tract (LRT) disease. Primary clinical endpoints will include prevention of hospitalization or medically attended lower respiratory tract infection (MALRI) in industrialized countries, and prevention of mortality and hospitalization in developing countries. Secondary goals would be: (I) prevention of medically attended LRT in young children; (II) prevention of hospitalization and mortality in the elderly; and (III) reduction of childhood wheezing, otitis media, and overall morbidity associated with RSV infection in children and adults (15).

Potential vaccine strategies to protect infants against RSV induced LRT disease include infant vaccination, maternal vaccination and vaccinating contacts of infants to block transmission. Therefore, the target populations for RSV vaccines are mainly five: (I) pregnant women; (II) infants <6 months of age; (III) infants and children >6 months to 2 years; (IV) young and school-age children; and (V) people >60 years of age (16).

The design of a pediatric RSV vaccine that is both safe and effective will have to take into account and obviate the mechanism by which FI-RSV caused ERD. Before embarking on human trials, the immunogenicity and safety of the vaccine candidates should be assessed in different animal models, including those that reproduce FI-RSV-associated immunopathology. Several animal models of ERD have been exploited, including mouse, cotton rat, non-human primates (NHPs) lamb, and calf models. Among these, cotton rats are more permissive to RSV infection and have a good standardization for pathologic scoring of alveolitis following FI-RSV immunization and viral challenge (17). The bovine model, though logistically more challenging and expensive to work with, is the most relevant to assess the RSV pathogenesis in humans. Experimental infection of calves with bovine RSV may cause nearly identical pathology in the bronchiolar epithelium, as is observed following natural infection with human RSV in infants and a consequently similar clinical disease (18). Although bovine RSV has only partial homology with human RSV, the F ectodomain is 90% identical and neutralizing antibodies to human RSV F can cross-neutralize bovine RSV, allowing indirect testing of human vaccines.

The risk of vaccine-induced ERD in seronegative infants may vary according to the different vaccine platform under consideration. Approaches based on protein subunits are more likely to induce a T-cell response pattern similar to FI-RSV because the antigen is processed through MHC class II pathways; though it is possible that the induction of highly functional antibodies could overcome the effect of a Th2-biased T-cell response. On the other hand, there is a general consensus on the fact that genetic vaccines, like viral vectored or nucleic acid-based vaccine platforms can mitigate the risk of vaccine-induced ERD since they behave biologically more like live virus vaccination in that the antigens are produced intracellularly, inducing CD8+ T-cells responses and skewing the adaptive immunity toward a Th1 phenotype. Based on these considerations, subunit proteins or protein-based nanoparticle vaccines are currently being proposed only for immunization of antigen-experienced individuals, particularly pregnant women, as a way of protecting neonates, and the elderly. These approaches are designed to primarily induce antibody-mediated protection, and are based on presenting the F glycoprotein (19).

Live-attenuated and chimeric live vectors have instead been advanced in antigen-naïve infants without being associated with ERD and have generally been safe and well tolerated. However the level of immunogenicity of these vaccines remains to be improved due to the great level of attenuation needed to prevent safety concerns (19).

Recombinant adenoviral vectors are a powerful technology for delivering vaccine antigens; their capacity

 

to infect cells and express encoded antigens that are shed into the extracellular milieu or directed to host intracellular processing pathways ensures highly efficient induction of both humoral and Th1 skewed, cytotoxic (CD8+) T-cell responses. This provides a key advantage over subunit vaccines, since CD8+ T-cells are critical for the elimination of intracellular pathogens. In addition, adenoviral vectors have intrinsic adjuvant properties, as they express diverse pathogen-associated molecular patterns which activate innate immunity. The main drawback of adeno vectored vaccines is that the transgene-specific response may be dampened by pre-existing or *de novo* adaptive immune responses to the vector itself. Strategies to overcome this issue include the use of higher doses, given the high tolerability profile of adenovirus vectors, and heterologous prime–boost regimens with adeno prime and other vaccine carriers for boost. In particular Modified Vaccinia Ankara (MVA) pans out as a potent booster component to enhance the magnitude of immune responses induced by priming with adenovirus vectors (20). Adenovirus based RSV vaccines have been extensively studied by many academic and industrial groups, exploiting human Ad5 (21), human rare serotypes Ad26 and Ad35 (22), chimpanzee PanAd3 (23) or gorilla GC46 (24) adenoviruses. Most of these RSV vaccine candidates expressing the viral F glycoprotein have been tested in mouse and cotton rat preclinical models, showing safety and protective efficacy. Notably, in one of these studies (23) also the safety and protective efficacy of a MVA vector expressing RSV antigens was tested in cotton rats showing protection from lung infection and pathology despite the lack of induction of neutralizing antibodies, thus suggesting that RSV-specific T-cells can mediate viral clearance in the absence of associated immunopathology.

So far, among the adeno-based vaccines and out of more than ten different clinical stage candidates, only one has been tested in the most relevant bovine model, showing safety and efficacy in antigen-naïve infant calves; this is based on a replication-defective chimpanzee Adenovirus, PanAd3-RSV, expressing three viral proteins: F, N and M2-1 (25). When testing the PanAd3-RSV vaccine in calves, Taylor *et al.* compared intranasal (IN) versus intramuscular (IM) route in different regimens of homologous and heterologous prime/boost to evaluate safety, immunogenicity and protective efficacy. From these studies it emerged that heterologous adeno prime/MVA boost vaccine regimens could completely prevent BRSV infection in the upper and lower respiratory tract of young seronegative calves. Efficient and safe protection from pulmonary infection and disease could also be achieved by a less complicated regimen based on a homologous intramuscular (IM) prime/boost with PanAd3-RSV (25). All vaccinated animals showed good level of serum neutralizing antibodies and T-cell responses that rapidly expanded after BRSV challenge. CD4+IFNγ+ appeared to be the most abundant RSV T-cells after vaccination, but BRSV infection boosted also a strong CD8+IFNγ+ responses.

This work provided evidence that a safe and protective RSV vaccine should be based on the ability to activate both B- and T-cells in a concerted manner. The inclusion in the vaccine antigen of the internal, conserved viral proteins N and M2-1 as source of T-cell epitopes turned out to be a valid strategy to increase the pool of RSV-specific T-cells, able mount a strong Th1 response upon virus encounter.

Such encouraging results supported the successful transition of this genetic vaccine technology for RSV from preclinical investigation to phase 1 safety and immunogenicity in humans. An open-label, dose escalation, phase 1 clinical trial was conducted in 42 healthy adults in which four different combinations of prime/boost vaccinations were investigated for safety and immunogenicity, including both IM and IN administration of the vectored vaccines (26). The phase 1 study showed that the vaccine was safe and well tolerated also when delivered by the IN route, and no vaccine-related serious adverse events occurred. Both intranasal and intramuscular administration of the vaccine led to increased neutralizing antibody titers above the high levels of pre-existing anti-RSV antibodies derived from repeated seasonal exposure to the virus. Circulating anti-F immunoglobulin G (IgG) and IgA antibody-secreting cells (ASCs) were observed after the IM prime and IM boost. RSV-specific T-cell responses were increased after the IM PanAd3-RSV prime and were most efficiently boosted by IM MVA-RSV. Interferon-γ (IFN-γ) secretion after boost was from both CD4+ and CD8+ T-cells, without detectable T helper cell 2 (Th2) cytokines that have been previously associated with immune pathogenesis following exposure to RSV after the formalin-inactivated RSV vaccine.

In conclusion, the genetic RSV vaccine was shown to be safe and immunogenic in healthy adults, warranting further clinical development in the elderly population. Although the overall burden of RSV in older adult is high, the rates of infection are relatively low (≈5%) and require the design of large clinical studies for testing the efficacy of vaccine candidates.

Clinical testing in adults can provide important

 

information about vaccine reactogenicity, but may not help to mitigate risk of ERD for RSV-naïve infants, due to the fact that adults were repeatedly infected throughout life and have strong immunity against RSV already at the time of vaccination.

Therefore, development of a pediatric RSV vaccine will require safety and immunogenicity studies to be performed by age de-escalation from adults to older RSV-primed children and finally young RSV naïve infants. Ultimately, the first clinical studies of a candidate genetic vaccine in seronegative infants will need to be carefully designed to rule out the risk of ERD before large pivotal phase 3 studies are conducted.

## Acknowledgements

None.

## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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**Cite this article as:** Vitelli A, Nicosia A. Virus *vs*. virus: adenovirus vectored vaccine to defeat respiratory syncytial virus. Ann Transl Med 2016;4(24):489. doi: 10.21037/atm.2016.12.08

 