Do recombinant-engineered nanoparticle vaccines hold promise for the prevention of respiratory syncytial virus?

“It holds a high promise that respiratory syncytial virus vaccines based on recombinant nanoparticles can be developed into a more effective vaccine than natural infection to prevent respiratory syncytial virus infection.”

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Safe & effective RSV vaccines: challenges

Human respiratory syncytial virus (RSV) can cause severe lung disease such as bronchiolitis and pneumonia in infants, young children, immunocompromised patients and in the elderly. Symptomatic infections are estimated to be 64 million annually worldwide, resulting in approximately 196,000 yearly fatalities [1]. During the 1960s, a formalin-inactivated RSV vaccine (FI-RSV) with alum adjuvant formulation was used to vaccinate young age children less than 6 months of age, which caused severe respiratory disease requiring hospitalizations and causing two deaths upon natural RSV infection [2]. Since this tragic failure of FI-RSV vaccine trials due to vaccine-enhanced disease (VED) upon infection, there is no licensed vaccine against RSV. In addition to VED, there are several hurdles for developing RSV vaccines. Severe respiratory RSV infection occurs in neonates and young infants. Some children and adults are re-infected every 3–10 years often with severe manifestation of sinus and/or asthma disease complications. Thus, the major challenges for developing RSV vaccines include protection of young infants, inability of natural infection-induced immunity to prevent reinfection and notorious legacy of VED.

Recombinant RSV nanoparticle vaccines in clinical trials

The RSV fusion (F) glycoprotein mediates viral entry into the target cells, is a target

by neutralizing antibodies and conserved in both RSV A and B strains. The monoclonal antibody palivizumab that has neutralizing activity by recognizing a single epitope on RSV F can prevent severe disease in high-risk infants by passive immunoprophylaxis. Purified RSV F protein-based vaccines were formulated with or without alum adjuvant, and administered to humans to assess their efficacies [3]. F protein vaccines were able to induce neutralizing antibodies without displaying adverse events in seropositive children. The best result was shown at a high dose without alum formulation. Approximately a four-fold increase in neutralizing antibody titers was observed in 50% vaccinated individuals. However, the efficacy of F protein vaccines on preventing RSV infection was not sufficiently high enough to justify continued investigation. Soluble RSV protein vaccines were demonstrated to induce pulmonary disease of VED after RSV infection in animal models [4,5].

A precursor (F0) of RSV F has been synthesized, posttranslationally cleaved at Arg109 and Arg136 by host furin proteases and processed into a shorter F2 polypeptide at the N-terminus linked by two disulfides to a longer F1 fragment with fusion and transmembrane domains. An RSV F nanoparticle vaccine [6] was developed by Novavax, a biotechnology company. First, RSV F was engineered to mutate the cleavage site (Arg136) near to the F1 N-terminus and to delete a part of the F1 hydrophobic fusion domain [6].

Young-Tae Lee

Institute of Biomedical Sciences,
Georgia State University, Atlanta,
GA 30303, USA

Young-Man Kwon

Institute of Biomedical Sciences,
Georgia State University, Atlanta,
GA 30303, USA

Sang-Moo Kang

Author for correspondence:
Institute of Biomedical Sciences,
Georgia State University, Atlanta,
GA 30303, USA
Tel.: +1 404 413 3588
Fax: +1 404 413 3580
skang24@gsu.edu

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Recombinant RSV F with near full-length disulfide-linked subunits of F2 and F1 was expressed on the surface of insect cells in a membrane-anchored homotrimer [6]. Insect cell membranes expressing recombinant RSV F were solubilized with nonionic detergent and subjected to a series of chromatography processes to purify RSV F-detergent complex fractions. Recombinant RSV F nanoparticles were formed into a pin-like rosette structure with approximately 40 nm after removal of the detergent [6].

“Recent progress in developing respiratory syncytial virus vaccines utilizing new advanced technologies is highly promising toward the goal of preventing respiratory syncytial virus disease.”

The Phase I clinical study was successfully carried out, demonstrating the safety and immunogenicity of the RSV F nanoparticle vaccine candidate in healthy adults 18–19 years of age [7]. An extended clinical study of RSV F nanoparticle vaccine with 350 healthy women (18–35 years) of childbearing age was recently published, demonstrating that the vaccine is safe, immunogenic and can reduce RSV infection [8]. Novavax released the data of a Phase II trial in press, reporting the safety and immunogenicity of RSV F nanoparticle vaccines (120 µg of RSV F with 0.4 mg alum adjuvant) in 50 healthy pregnant women in their third trimester. Maternally transferred RSV specific and neutralizing antibodies with a half-life between 30 and 41 days were observed in infants born to vaccinated mothers with RSV F nanoparticle vaccine. Also, Novavax announced the grant from the Bill & Melinda Gates Foundation to support the Phase III clinical trial studies of the RSV F vaccine in pregnant women for maternal immunization, expecting the protection of young infants from RSV infection.

Recombinant virus-like nanoparticle vaccines presenting RSV glycoproteins in preclinical studies

Virus-like nanoparticles (VLPs) can be self-assembled in cells expressing viral structural proteins and present repeating protein antigen arrays similar to those of virus in structure and morphology. The size of VLPs is in a range of approximately 100 nm depending on the viral core proteins driving the VLP assembly and formation. Newcastle disease virus (NDV) or influenza virus core proteins have been utilized to present RSV F and/or G glycoproteins on the VLP membrane surfaces because of poor capability of RSV structural proteins to form VLPs. For efficient incorporation into NDV-VLPs, chimeric constructs were engineered

by fusing the sequences encoding the ectodomains of RSV G or F to the transmembrane and cytoplasmic domains of the NDV glycoproteins [9]. Transfection of avian cells with DNA plasmids encoding NDV structural proteins (NP, M) and RSV chimeric G and/or F glycoproteins generated VLPs containing the RSV G or both F and G proteins [9,10]. Chimeric RSV–NDV VLPs were shown to be more effective than live RSV in eliciting RSV-neutralizing antibodies and high ratios of IgG2a/IgG1 isotypes indicating T-helper type 1 (Th1) immune responses, and inhibiting viral replication in mice upon challenge. VLP-associated VED as assessed by pulmonary histopathological analysis was not observed in comparison with FI-RSV in BALB/c mice. Neutralizing antibodies induced by chimeric RSV–NDV VLPs were lasted for 14 months, whereas those by live RSV declined after 3 months [11]. Also, RSV–NDV VLPs that were engineered to present a prefusion form of RSV F effectively induced neutralizing antibodies after immunization of mice or cotton rats in the absence of adjuvants and confer protection without VED upon RSV challenge [12,13].

Coexpression of RSV F or G and influenza virus M1 matrix protein in insect cells using the baculovirus expression system resulted in efficient release of VLPs presenting RSV F or G, which are effective in inducing Th1-type antibody responses and inhibiting lung viral replication in mice [14]. A combination of RSV F and G VLPs plus F DNA plasmids was effective in inducing RSV-neutralizing antibodies, Th1-type IFN-γ secreting CD8 T cells and CD11c⁺ phenotypic cells in the airways and preventing eosinophilic pulmonary histopathology after RSV challenge of mice, even better than the immunity by live RSV reinfections [15,16]. Mice that were vaccinated 12 months earlier with RSV F and G VLPs maintained protective immunity in contrast to FI-RSV causing VED characterized by eosinophils, IL-4-producing CD4 T cells, proinflammatory cytokines, chemokines and plasmacytoid dendritic cells (DCs) [17]. Furthermore, live RSV reinfections induced IFN-γ and tumor necrosis factor-α producing CD4 T cells and CD11b⁺ DCs at high levels, which might be contributing to RSV disease and pulmonary VED despite lung viral clearance after challenge [18]. In contrast, RSV F VLP immunization of mice induced CD8α⁺ DCs and IFN-γ producing CD8 T-cell responses in the lungs, preventing pulmonary histopathology of VED [18]. Thus, F VLP nanoparticle vaccination can induce distinct innate and adaptive cellular subsets preventing pulmonary VED in mice, which are different from FI-RSV vaccination and live RSV reinfections, after RSV challenge.

Future prospects

Recent progress in developing RSV vaccines utilizing new advanced technologies is highly promising toward the goal of preventing RSV disease. The Novavax RSV F nanoparticle vaccine candidate produced in insect cells is advancing to the Phase III clinical trial of maternal immunization in pregnant women, expecting protection against RSV in neonates and young infants. The RSV F nanoparticle vaccine can be safer and more effective in eliciting palivizumab-like RSV-neutralizing antibodies than FI-RSV or live RSV.

There is still a challenge to induce immunity in young infants and children after 3 months of age when maternal antibodies wane below the protection limit. It needs to be investigated whether maternal antibodies or pre-existing immunity may have inhibitory effects on the efficacy of active RSV vaccination in young children. Use of the alum adjuvant formulation in the RSV F vaccination may have a safety concern due to its biased induction of Th2-type immune responses associated with pulmonary VED in seronegative children upon RSV infection.

Recombinant-engineered RSV VLP vaccines do not require adjuvants for eliciting antibodies to a level of protection. Also, VLPs presenting a stabilized prefusion form of RSV F appear to be quite promising candidates. Manufacturing and regulatory processes should be followed to advance VLP-based RSV vaccines for clinical trials. It holds a high promise that RSV vaccines based on recombinant nanoparticles can be developed into a more effective vaccine than natural infection to prevent RSV infection.

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