Getting at the crux of the antigen

By Tracey Baas, Senior Editor

Novartis AG researchers have used structure-based antigen design to rationally optimize vaccines for respiratory syncytial virus¹ and Group B *Streptococcus*.² Although the strategy itself is not new, the work serves as proof of concept for its application to the development of vaccines designed to be more effective than vaccines that use native viral or bacterial macromolecules to elicit immune responses.

"At Novartis, we use structural vaccinology to design and optimize all new antigens for our vaccine programs, with RSV and GBS being two examples of this technology at work," said Philip Dormitzer, head of the viral advanced programs team and senior project leader of viral vaccine research at Novartis and a coauthor of the respiratory syncytial virus (RSV) paper. "We have several products across the phases of development."

For its vaccine antigen, the RSV team set out to optimize the RSV fusion glycoprotein (RSV F), a membrane-anchored glycoprotein that mediates viral entry into host cells. The target also mediates fusion of the membrane of infected cells with adjacent cells to form the syncytia characteristic of the disease.

The goal was to improve the stability, purity, reproducibility and potency of current RSV F-based vaccine candidates.

AstraZeneca plc markets the RSV F-targeting antibody Synagis palivizumab to prevent RSV infection in high-risk infants. The pharma's MedImmune LLC unit is developing motavizumab, a humanized RSV F-targeting antibody that is in Phase II testing to treat RSV. MedImmune discontinued its development for RSV prophylaxis and withdrew a BLA in the indication last December following a request by FDA for a further trial.

Novartis' Group B *Streptococcus* (GBS) team set out to show that the rational design strategy is applicable in the bacterial space. Thus, the researchers designed a vaccine candidate targeting the six known variants of the protective D3 domain of the pilus backbone protein 2a (BP-2a) found in different GBS strains.

Both groups published their results in the *Proceedings of the National Academy of Sciences*.

On the RSV front

"Because of the expense and other limitations of mAb prophylaxis, there is a very large unmet

need for an RSV vaccine in spite of the currently licensed antibodies. While Synagis exists as a prophylactic for the highest risk infants, a vaccine could protect all infants," said Dormitzer.

RSV F exists in two distinct conformations: a lollipop-shaped prefusion form and a crutch-shaped postfusion form. The prefusion form readily rearranges into the lower energy postfusion form, which aggregates due to exposure of a hydrophobic fusion peptide.

By deleting the RSV fusion peptide, transmembrane region and cytoplasmic domain, the team designed an antigen resembling postfusion RSV F that could be produced in a baculovirus and insect cell expression system and that allowed for crystallization rather than aggregation. The construct formed a stable, nonaggregating trimer, allowing the group to solve its crystal structure at 3.2 Å resolution for the first time.

Previously, the main RSV F structural information that could be used for vaccine design was based on homology modeling³⁻⁶ and antibody-RSV peptide epitope complexes.⁷ Those data implied the major neutralization epitope was buried in the RSV F trimer. The new crystal structure reveals that the epitope is exposed at the RSV F surface, where it could elicit protective, neutralizing antibodies after immunization.

Cotton rats injected with the alum-adsorbed postfusion RSV F trimer complex had protective, RSV-neutralizing antibodies after immunization and undetectable pulmonary viral loads following intranasal RSV challenge compared with nonimmunized rats.

Competition ELISA showed the sera of mice immunized with the alum-adsorbed postfusion RSV F antigen competitively inhibited Synagis binding, indicating that immunization with the engineered antigen elicited antibodies similar to those that protect high-risk newborns, as predicted by examination of the structure.

"There have been some trials with other modified RSV F preparations that were probably in the postfusion conformation" said José Melero, director of the National Microbiology Center at Carlos III Health Institute. "They were well tolerated and no sign of enhanced disease was observed, but they also showed no significant protection. Therefore, safety and efficacy are critical steps to be tested with any new prototype of RSV vaccine."

The key thus appears to be showing that Novartis' structure-based work has created a trimer that will be more potent than previously tested RSV F preparations.

He suggested that "the soluble RSV F trimer seems very robust, which could facilitate its industrial-scale production and purification for vaccine purposes. Higher doses of the soluble RSV F trimer could therefore be tested, which could not be done with previous RSV F preparations, to determine whether they could induce protection in humans without adverse effects."

Jason McLellan, postdoctoral fellow at NIH's National Institute of Allergy and Infectious

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Diseases, said he is interested to see how the stabilized postfusion construct compares to other forms of RSV F in terms of potency.

"Most forms of RSV F elicit high titers of neutralizing antibodies. Since the postfusion form of RSV F is the most stable, it's likely that researchers have been immunizing with some form of the postfusion F protein for years," McLellan said.

Closing in on GBS

The other Novartis team used the same structural approach to design 6xD3, a vaccine that protects against all type 2a pilus-carrying GBS strains. The structure-based 6xD3 antigen is a chimeric protein that contains six different protective D3 domains separated by four-mer peptide spacers. Each of the six domains represents a GBS variant of BP-2a.

Two other types of backbone proteins exist for GBS strains: type 1 and type 2b. These have only one variant each.

In mice, maternal 6xD3 immunization protected about 76% of pups from various type 2a pilus-carrying GBS strains. Maternal saline immunization protected only about 26% of the pups.

6xD3 is in preclinical development. Novartis' next steps include developing a universal protein-based vaccine against GBS. To do so, the company will need to engineer a product that also targets BP-1 and BP-2b.

The pharma is tackling that goal via two approaches. The first is developing a vaccine cocktail formulation containing the 6xD3 chimeric protein, BP-1 protein and BP-2b protein. The second is developing a larger chimeric protein incorporating the 6xD3 protein, BP-1 and BP-2b.

"We are working on both strategies, and we plan to move forward

with the simplest solution," Rino Rappuoli, global head of vaccines research at Novartis Vaccines and Diagnostics, told *SciBX*.

Novartis has filed patent applications based on the RSV and GBS work. Novartis' GBS team collaborated with researchers from the **University of Milan**, who solved the 3D structure of one of the BP-2a variants.

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