Besieging RSV

By Michael J. Haas, Senior Writer

A team of U.S. and Chilean researchers has reported that a vaccine derived from bacillus Calmette-Guérin could prevent respiratory syncytial virus infection in infants and children.[1] It's unlikely the vaccine would take market share from the lone marketed RSV antibody, Synagis palivizumab, because it would target different pediatric populations.

By the age of two, most children have been infected by RSV at least once, and in most cases the infection is almost indistinguishable from a common cold. But in infants younger than six months, RSV is a leading cause of hospitalization, especially in infants born prematurely, born close to the annual RSV season and/or born with chronic heart or lung disease.

Synagis, a first-generation anti-RSV antibody marketed by **AstraZeneca plc**'s **MedImmune Inc.** business unit, is the only prophylactic RSV therapy on the market. The high cost of the drug has limited its use to premature infants and precluded its application in full-term infants and young children.[2] Despite those limitations, Synagis sales in the first nine months of 2008 were $724 million.

Development of a vaccine for use in the general pediatric population has faced other roadblocks. RSV infection is known to induce only short-lived immunity against subsequent infections, making it challenging to develop a vaccine that is more strongly immunogenic than the virus itself.

In the 1960s, a vaccine based on formalin-inactivated RSV actually exacerbated infection and even caused some deaths among immunized children for reasons that are still poorly understood.[3,4] Consequently, there are only two vaccines and a handful of antiviral agents in commercial development to prevent or treat RSV (*see* **Table 1, "RSV pipeline"**).

One prevailing theory has been that the formalin-inactivated vaccine induced a T helper type 2 cell (T_h2)-based immune response rather than a protective T_h1-based response, resulting in lung inflammation and lung injury.

Trying BCG

In an article in the *Proceedings of the National Academy of Sciences*, the research team hypothesized that a vaccine capable of inducing a T_h1 response might confer better protection against RSV than the formalin-inactivated vaccine. The team zeroed in on BCG, a *Mycobacterium bovis* strain known to induce an effective T_h1 response against tuberculosis.

First, the researchers engineered BCG to express one of two RSV antigens, N or M2. They inoculated mice with one of two vaccines, BCG-N or BCG-M2, and challenged the mice with RSV 21 days later. Mice given either vaccine did not exhibit two symptoms characteristic of RSV infection: rapid weight loss or signs of pulmonary inflammation. Examination of airway samples and lung tissue samples showed that the immunized mice had lower viral loads than controls and fewer signs of pulmonary inflammation such as neutrophil infiltration.

T cells isolated from spleens of immunized mice responded to stimulation with N or M2 antigen by secreting IL-2 and interferon-γ (IFN-γ), which is indicative of a T_h1 response, but did not secrete cytokines associated with a T_h2 response, such as IL-4 or IL-10. Furthermore, the team did not detect antibodies specific for N or M2 in the

Table 1. RSV pipeline. Only one drug is marketed to prevent serious lower respiratory tract disease caused by pediatric respiratory syncytial virus (RSV) infection, whereas at least six other compounds are in development to prevent serious respiratory illness caused by the virus and/or to treat the infection.

Company	Product	Description	Status
MedImmune Inc. unit of **AstraZeneca plc** (LSE:AZN; NYSE:AZN)/**Abbott Laboratories** (NYSE:ABT)	Synagis palivizumab	Prophylactic humanized mAb against RSV	Marketed
MedImmune	Motavizumab	Prophylactic humanized RSV antibody	Resubmit BLA 1H09
Alnylam Pharmaceuticals Inc. (NASDAQ:ALNY)/**Kyowa Hakko Kirin Co. Ltd.** (Tokyo:4151)	ALN-RSV01	Therapeutic RSV-specific small interfering RNA	Phase II
MedImmune	MEDI-559	Prophylactic live attenuated RSV vaccine	Phase I/II
MedImmune	MEDI-534	Prophylactic bovine parainfluenza virus Type III (PIV-3) expressing the native or soluble fusion protein of RSV	Phase I/II
Biota Holdings Ltd. (ASX:BTA)/MedImmune	BTA9881	Therapeutic small molecule fusion inhibitor	Phase I
Symphogen A/S	Sym003	Prophylactic recombinant human polyclonal antibody against RSV	Preclinical

Source: BCIQ: BioCentury Online Intelligence

immunized mice, indicating that the vaccine had not induced B cells to produce antibodies.

Additional experiments involving adoptive transfer of T cells from immunized mice to RSV-naïve mice confirmed the T_h1 response.

The team concluded that a BCG-based vaccine could protect against RSV via a T_h1 response and wrote that such a vaccine should be safe for use in infants, as BCG has been used for decades to prevent TB in infants, children and adults worldwide.

In the U.S., the **Centers for Disease Control and Prevention** recommends the BCG-based TB vaccine for use only in healthcare workers and children who are continually exposed to TB-infected individuals (http://www.cdc.gov/tb/pubs/corecurr/Chapter9/Chapter_9_Recommendations.htm). The vaccine has variable effectiveness in adolescents and adults and can cause a false positive in the tuberculin skin test, a diagnostic for TB infection.

The *PNAS* team was led by Alexis Kalergis, assistant professor of molecular immunology at **Pontifical Catholic University of Chile** (PUC), and included scientists from PUC, **Jacobi Medical Center**, **Albert Einstein College of Medicine** and the **Andrés Bello University**.

Response in Ab-sentia

Companies contacted by *SciBX* said it was not yet clear whether inducing a T_h1-type response—but not antibodies—would be sufficient to protect humans from RSV infection.

Jessie Groothuis, VP of the RSV franchise for medical affairs at MedImmune, agreed that the research team's hypothesis was sound. "If a BCG vaccine can push the immune response from T_h2 to T_h1, that could be a good thing," she said.

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But Groothuis noted that mouse models are not ideal, even though they are widely used for vaccine research. Mice are not as susceptible to RSV infection as humans, and thus must be given disproportionately large doses of virus to induce disease symptoms. "The next step would be to get the vaccine into nonhuman primates before more could be said about how promising it is," she said.

MedImmune's Numax motavizumab, a humanized RSV antibody, is under regulatory review to prevent RSV infection in at-risk infants six months and younger. MedImmune is also developing two vaccines to prevent serious respiratory illness caused by RSV infection. MEDI-559, a live attenuated RSV vaccine, is in Phase I/II trials, and MEDI-534, a bovine parainfluenza virus Type III (PIV3) vaccine expressing RSV antigens, is in a Phase I study.

Sonya Cyr, head of preclinical RSV and cell-mediated immunology at GlaxoSmithKline Biologicals North America, said the T_h2 immune response played a role in the failure of the formalin-inactivated vaccine, but added that the failure probably involved additional factors.

"The focus on T cells is not the complete story," she said. "The explanation could be that the vaccine induced low-affinity antibodies or, as a recent *Nature Medicine* paper suggests, the vaccine induced poor TLR stimulation."[5]

GlaxoSmithKline Biologicals, a business unit of **GlaxoSmithKline plc**, markets vaccines to protect against influenza, hepatitis A and other infections. The unit is also developing vaccines against meningitis, HPV and other infectious diseases.

Groothuis said it was not clear to her why the team chose N and M2 antigens rather than the more frequently studied F and G antigens found on the surface of the virus.

Kalergis was not available to comment. But GSK's Cyr said the choice of these antigens was logical and consistent with the team's goal of inducing T cell–based immunity, because N and M2 have known T cell epitopes.

On the other hand, she said it was unclear what markers of protection could be used in clinical trials without antibodies.

"Antibodies are the only known correlative to protection in humans," said Cyr. She added that other clinical markers, such as hospitalization rates or counts of antigen-specific T cells, would be only indirect measures of protection.

Indeed, according to Cyr, developing a vaccine that did not induce antibodies would be a risky strategy, given that the one approved RSV prophylactic demonstrates that antibodies play an important role in protection against infection.

Thus, she said a vaccine that targeted surface antigens to elicit high-affinity antibodies would be preferable to a vaccine that induced only T cell–based protection.

Steve Bende, president and CEO of **Bacilligen Inc.**, said that if antibodies were important to protecting humans against RSV, "you might need to add a boost inoculation of recombinant RSV proteins" to a BCG-based prime inoculation to stimulate antibody production.

Bacilligen's lead program, a next-generation recombinant version of BCG, is in preclinical development as a therapeutic—not a vaccine vector—to treat superficial bladder cancer.

Cyr cautioned that BCG as a vaccine vector has a history of inconsistency and low effectiveness. "The BCG vaccine has been attributed to the reduction of TB-related hospitalization rates in some parts of the developing world," she said, but "most experts agree that it is not very effective," which might make a BCG-based vaccine against RSV a tough sell in the developed world.

Kid stuff

Even if the BCG-based approach proves effective against RSV, it would probably not compete with Synagis because an RSV vaccine would target different populations than the anti-RSV antibody.

John Haurum, CSO of **Symphogen A/S**, said treating newborns, Synagis' target population, would not be the best strategy for an RSV vaccine because it is difficult to raise an immune response with a vaccine in infants younger than about six months.

Cyr agreed, noting growing evidence that maternal antibodies can interfere with an infant's response to a vaccine.[6] "It is still not clear how to bypass the issues of maternal antibodies interfering with infant immune response, except to vaccinate pregnant mothers," which presents its own set of challenges, she said.

Instead, both Haurum and Cyr suggested that an RSV vaccine should target healthy infants and children between six months and five years of age to promote herd immunity, which eventually could indirectly lower the risk of RSV infection in premature infants.

Symphogen's Sym003, a recombinant human polyclonal antibody against RSV, is in preclinical development to prevent serious lower

respiratory tract disease caused by RSV in pediatric patients at high risk of RSV disease.

MedImmune's two RSV vaccines will target two groups that do not include at-risk infants: the elderly, in whom severe RSV disease is now known to be more prevalent than previously suspected; and healthy, full-term newborns up to six months old.

Groothuis thus would not entirely rule out the possibility that a vaccine could be effective in newborns, as "the amount of maternal antibodies a healthy newborn has can vary widely."

She also said an RSV vaccine would not supplant the need for protecting premature infants. "The market for therapies targeting the at-risk population is not going away anytime soon," she said.

The findings reported in *PNAS* were patented both in Chile and internationally by PUC in 2008 and are available for licensing, according to Catalina Bay-Schmith, technology transfer manager of **Office for Research Transfer, Chile** (OTRI Chile). The patents cover vaccines using BCG or related *Mycobacterium* vectors that express RSV antigens.

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