Synthetic dual-action vaccines

By Lev Osherovich, Senior Writer

U.S. and Canadian researchers have developed a technique to produce fully synthetic vaccines and have mouse data showing the prevention of candidiasis, a range of oral, vaginal and systemic yeast infections.¹ Although a candidiasis vaccine would have limited commercial prospects, **Wellstat Therapeutics Inc.** says the data provide proof of concept for the technology. The company has in-licensed relevant facets of the synthetic vaccine technology and has used it to move vaccines into preclinical development for other pathogens.

Although the next academic step is to test scaled-up versions of the researchers' vaccine, one question for drug developers will be whether the technique can be applied to more complex pathogens.

The study, published in the *Proceedings of the National Academy of Sciences*, was conducted by a team led by Jim Cutler, professor of pediatrics at **Louisiana State University Health Sciences Center** and associate director of the Research Institute for Children at **Children's Hospital, New Orleans**.

The Louisiana State University researchers developed six vaccines that all consisted of two antigens joined by a flexible linker molecule. The two antigens were derived from *Candida albicans*, the fungus that causes candidiasis, and mimicked a cell-wall carbohydrate and a peptide derived from a virulence factor. In every case, the carbohydrate moiety was a β -1,2-mannopyranosyl trisaccharide known to elicit the production of antibodies in mice²—each vaccine contained a different virulence peptide.

Unlike most vaccines, which are made from cellular fractions or using recombinant expression systems, all the components of Cutler's vaccines were synthesized and assembled *in vitro*.

The team delivered the vaccines to dendritic cells (DCs) obtained from mouse bone marrow. DCs gather antigens and present them to acquired immune system players such as B cells.

After a week, the treated DCs were reintroduced into mice, which were then given two weeks to mount an acquired immune response. Compared with mock-treated controls, mice that received transplants of DCs treated with each of the six vaccines showed high levels of reactive serum against each vaccine component—both peptide and sugar.

In a direct immunization experiment with vaccinated DCs, the

resulting antibodies subsequently protected mice from fungal infection. Five of six vaccines led to lower fungal titers in the kidneys of mice exposed to *Candida*. Also, 80–100% of mice receiving the most promising candidate vaccines were alive after 120 days compared with none for the controls.

Moreover, in a passive immunization experiment, serum from the first set of mice protected a second batch of nonimmunized mice against disseminated candidiasis. This suggests that serum-borne antibodies, rather than T cells, are primarily responsible for the protective effects of vaccination. However, Cutler told *SciBX* that it's unclear whether the most protective antibodies are against the carbohydrate, the peptide or both.

Cutler attributed the success of the vaccines to the synergistic effect of raising multiple types of antibodies. Previous synthetic vaccines for other pathogens used immunologically inert peptide carriers³ and primarily led to polysaccharide-specific antibodies.

"We have found that some of our vaccine constructs prove a double hit for the immune system," he said. "Not only is the carbohydrate immunogenic, but the peptide provides an additional level of protection."

The next step for Cutler's group is to scale up the synthesis of the most highly protective lead candidate for testing in a monkey model of *Candida* infection.

Candida candor

Cutler's team chose *Candida* for proof of concept because of their experience in characterizing immunogenic polysaccharides from the fungus. But a candidiasis vaccine itself is

unlikely to have commercial utility because most yeast infections are readily treatable with over-the-counter antifungals.

Moreover, although disseminated candidiasis is life threatening for immunocompromised subjects, a prophylactic vaccine may be unable to elicit an immune response in such patients.

Nevertheless, Wellstat views the work published in *PNAS* as proof of concept for the approach. The company has licensed patents from separate companies founded by Cutler and David Bundle, a professor of chemistry at the **University of Alberta** and a coauthor of the *PNAS* paper.

Cutler is a founder of vaccine company **LigoCyte Pharmaceuticals Inc.** The company in-licensed polysaccharide-based *Candida* vaccine IP in the late 1990s from **Montana State University**, where he was then a professor of microbiology. In 2006, LigoCyte out-licensed the project to Wellstat (then called BioVeris Inc.), in order to focus on viral indications.

Wellstat also in-licensed IP from **TheraCarb Inc.**, a carbohydrate chemistry company cofounded by Bundle, that provided some of the synthetic tools used to make the vaccines.

Francis Michon, Wellstat's general manager of vaccines, told *SciBX* that the key selling point for the technology is the ability to develop synthetic conjugated vaccines against a variety of pathogens. Such vaccines should offer several advantages over recombinant or fermentation-based production methods, he said.

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—Jim Cutler,
**Louisiana State University
Health Sciences Center**

Marketed conjugate vaccines such as **Merck & Co. Inc.**'s Pneumovax pneumococcal vaccine or **Wyeth's** Prevnar PCV7 for *Pneumococcus* typically contain polysaccharides from cultured pathogens coupled to recombinant carrier proteins. Michon thinks fully synthetic vaccines should have better batch-to-batch similarity, which could lead to more consistent biological responses in trials.

As a consequence, he suggested that "there may be an easier regulatory route, and you may be able to manufacture large amounts" of the vaccine using total chemical synthesis.

Michon said Wellstat's synthetic conjugate vaccine programs range from early discovery to late preclinical development and are focused on a "wide range of bacterial vaccines." The most advanced programs target *Neisseria meningitides* and group B *Streptococcus*.

All of these pathogens are amenable to the synthetic conjugate vaccine strategy, he said.

Michael Wacker, CTO of conjugate vaccine maker **GlycoVaxyn AG**, sees things differently. Wacker told *SciBX* that the fully synthetic method is most likely suitable only for simple trisaccharides such as those made by *Candida*; the method will be more challenging to apply to the complex sugar antigens of most bacterial pathogens.

"Bacteria have a huge variety of sugars, so it's not so straightforward to synthesize all of the different precursors," he said.

GlycoVaxyn is developing vaccines against bacterial pathogens using a recombinant expression system in which both the carbohydrate

and peptide parts of the vaccine are made and linked together in *E. coli*. The company's peptides act as carriers to activate immunity against the polysaccharide, according to Wacker.

Wacker expects GlycoVaxyn's lead candidate vaccine against *Shigella*, an enteric pathogen, to enter Phase I testing in 2009.

Wacker noted that biological expression systems have been used for recombinant vaccines for decades, so there are few safety concerns about glycoconjugates from recombinant bacteria. In contrast, he said, the safety of synthetic polysaccharides for vaccination is still unknown.

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COMPANIES AND INSTITUTIONS MENTIONED

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