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Innovations

# Ancora Cooks with Carbs Synthesizing Carbohydrate Vaccines

In the 200 years since Edward Jenner showed that immunity to smallpox could be induced by inoculating people with cowpox, vaccines have been the workhorses of public health. Diseases such as measles, diphtheria, rubella, and polio are barely encountered any more in developed countries.

Traditionally, vaccines have been made from dead or attenuated pathogens, but in some cases where this approach is unsuitable, an antigen isolated from the pathogen surface is used to induce immunity. Most of the antigens that are selected are proteins, because in most cases proteins are the most abundant antigens expressed on the pathogen surface, and they induce a strong immune response in humans. But since antigenic proteins, like all proteins, are directly encoded by a gene, any mutation in the gene will result in a change in the antigen. When this happens, the resulting antigen is treated by the immune system as a new antigen, and any former immunity will be lost, as in the cases of the common cold or HIV viruses. Pathogens such as protozoans that have a complex life cycle shift their surface proteins (in some cases, as often as every two weeks) as they evolve, making it hard for the immune system to fight them or for any long-term immunity to be established or artificially induced.

For these reasons, immunologists have turned to other antigens that are less susceptible to change by mutation and are rarely shifted in the life cycle of pathogens. Such antigens are complex carbohydrates. Carbohydrate antigens are synthesized and expressed via pathways involving at least one enzyme, and, although the enzyme is subject to mutation, most nonlethal mutations result in a functional enzyme. Thus, the carbohydrate that it builds will not be altered.

However, carbohydrate antigens pose certain difficulties. In most cases, their surface densities are small, which makes isolating them from the organism in sufficient quantities unfeasible; most of these antigenic carbohydrates are complex structures of polysaccharide chains that are tethered to a cell wall protein or phospholipids. Until recently, it was difficult to synthesize them, and to obtain reasonable yields. Furthermore, carbohydrate antigens do not stimulate the human immune system as readily as do proteins, and they need to be conjugated with an adjuvant to induce immunity, especially in infants.

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For these reasons, even though research on carbohydrate-based vaccines at the Rockefeller University was initiated as early as the 1920s, only a few have met with success. These include vaccines against Haemophilus influenzae (HiB), a disease that infects 3 million people and causes 400,000 to 700,000 deaths annually, particularly of infants aged 4 to 18 months. The first carbohydrate HiB vaccines from bacterial isolates were approved in the U.S. in the mid-1980s [1]. A completely synthetic version was approved for use in Cuba in 2004 [2]. Other carbohydrate-based vaccines, such as MenC, from bacterial isolates exist for meningococcal group C (a MenB vaccine is in phase II trials) to vaccinate against meningitis, a disease that affects 120,000 people annually, with a mortality rate of between 10 and 15 percent. Meningococci and the HiB pathogen both belong to a family of bacteria that express a large amount of polysaccharides on their cell capsules.

More recent work on synthesis of complex carbohydrates by Professor Samuel Danishefsky's group at Columbia University and Memorial Sloan-Kettering Cancer Center (MSKCC) and others has vastly extended the possibilities for synthesizing complex carbohydrates. These new synthetic paths may open new avenues for vaccine development. "I don't think anyone says that carbohydrate vaccines in principle ought to be better than a peptide vaccine," said Danishefsky, "… the carbohydrate area is an overlooked area. And it can be attacked because of major advances in chemistry."

## Evasive Action

Leishmaniasis is a disfiguring disease caused by a protozoan (Leishmania), spread by the female sandfly, that affects 12 million people worldwide. Visceral leishmanaisis, the fatal variant, is estimated to affect at least 500,000 people annually. A Leishmania protozoan expresses different protein antigens as it evolves through its life cycle. As a result, it is hard for the immune system to effectively resist the pathogen, and conventional vaccines have failed to induce substantial immunity. Malaria is a deadly disease that is caused by another protozoan with a complex life cycle for which no effective vaccine exists to date. Because the carbohydrate antigens on the surfaces of these parasites do not shift as often as the protein antigens, they are good candidates on which to base a vaccine.

Dr. Peter H. Seeberger, formerly a post-doc in the Danishefsky laboratory and now at the Swiss Federal Institute of Technology (ETH), has been working for years with

immunologists to develop fully synthetic, carbohydrate-based vaccines. His expertise in synthesizing complex carbohydrates played a major role in efforts to develop vaccines against leishmaniasis and malaria, both currently in preclinical stages.

The malaria vaccine, developed in collaboration with microbiologist Louis Schofield of the Walter and Eliza Hall Institute of Medical Research in Australia, blocks the toxins secreted by the micro-organism. It does not aim at preventing the disease, but rather at preventing its acute symptoms and reducing mortality. Currently it is being tested "in higher-order animals," says Seeberger.

This year, Seeberger led a multidisciplinary group of chemists and immunologists from Pevion, a Swiss biotech, and the Swiss Tropical Institute in Basel that announced a two-part synthetic vaccine candidate for visceral leishmaniasis [3]. Seeberger's group was responsible for finding a route for the synthesis of the complex carbohydrate antigen, the key element in the vaccine. In order to get a sufficient immune response, the carbohydrate antigen was conjugated to the empty shell of an influenza virus that served as an adjuvant to stimulate the body's immune system.

**Ancora's Carbohydrate Factory**

Ancora Pharmaceuticals (www.ancorapharma.com) was started in 2002 by Seeberger and others to develop a synthesis platform for complex carbohydrates after they won the Massachusetts Institute of Technology's 50K entrepreneurial competition. The company, numbering less than ten people, is located in Medford, Massachusetts and occupies space vacated by Kinetix and Nanosys. "Distinguished predecessors," said John Pena, PhD, president. According to Pena, Ancora has a lead candidate to treat malaria in preclinical studies. Vaccine candidates targeted against leishmaniasis and tuberculosis will be in preclinical studies by the end of the year. The company is also eyeing anticoagulants and vaccines for autoimmune disorders, as well as pathogens, such as anthrax, used in biowarfare. The malaria program is funded by a $3.3 million National Institutes of Health (NIH) SBIR grant. The NIH is also providing support for their tuberculosis program, and the company has a round of private investment.

"Ancora has a platform technology which we developed in my lab when I was at still at MIT," said Seeberger. "This is a way of automated oligosaccharide synthesis that gives us access to molecules about 500 times faster than previously. It is about a day instead of about a year to a year and a half. Ancora has also in-house capabilities to make much larger quantities." According to Seeberger, although the synthesizer typically makes small quantities, Ancora can also produce more, such as tens of grams to kilograms of the malaria vaccine candidate.

Seeberger notes that a total laboratory synthesis procedure has to be completed before scaling up and automation can be implemented. Among the many challenges in synthesizing carbohydrates is that the monomers are complex and can react through many different sites. It is unclear whether it is possible to develop an automated, universal method that would be appropriate for a large variety of carbohydrates. The synthetic methodology employed by Ancora, adopted from automated synthesis used to manufacture oligonucleotides, enzymes, peptides, and certain proteins, is to assemble a library of intermediate species that serve as building blocks for many complex carbohydrates and to combine them to assemble the desired molecule, rather like building a prefab house of modular components. If Ancora can find a way of automating a substantial number of key candidates, it will indeed be a major achievement.

Vaccines in general, and vaccines for developing countries, target a large market, but it is not necessarily a lucrative one. Consequently, "Ancora has a much broader platform that also does carbohydrate arrays," Seeberger said. "The synthesis platform is really the base of the company. The vaccine application is one big application that Ancora is obviously doing."

There is interest from unnamed large pharmas for the leishmaniasis vaccine. "Initially, veterinary vaccines may be the way to go," said Seeberger. "The tests are relatively expensive. It is cheaper to test in dogs. The next step if these veterinary vaccines work well [is to] go back into humans."

**Cancer Vaccines**

One of the many approaches to treating cancer is using a vaccine to induce the immune system to fight the disease. These vaccines, in an attempt to avoid recurrence of the tumor, are aimed predominantly at people who have undergone cancer treatment. Targeting the patterns of carbohydrate antigens that are overexpressed by some cancer cells is one promising strategy. One example is a three-part cancer vaccine that is in development by Professor Geert-Jan Boons (http://cell.ccrc.uga.edu/%7egjboons/boons/Home.htm) of the Complex Carbohydrate Research Center of the University of Georgia, Athens and uses a peptide antigen as an adjuvant. Samuel Danishefsky's laboratory is also working on synthetic carbohydrate vaccines for cancer including Globo-H, a unimolecular multivalent candidate targeting breast cancer. Globo-H constitutes a single glycopeptide that contains five different cancer antigens [4].

Danishefsky is also a cofounder of Optimer Pharmaceuticals (www.optimerpharma.com), along with Dr. Chi-Huey Wong, professor of chemistry at The Scripps Research Institute in La Jolla, California and Michael N. Chang, PhD. The San Diego company, founded in 1998, licensed carbohydrate cancer vaccines from Danishefsky's laboratory. Optimer streamlines oligosaccharide synthesis by avoiding the dreaded chromatography stage of separation through the development of a "one-pot synthesis" to produce the carbohydrate antigen.

Other carbohydrate cancer vaccines in clinical trials are being developed by New York-based Progenics Pharmaceuticals (www.progenics.com) and are currently part of a multiyear study that was initiated by MSKCC and carried out in Europe. Biomira (www.biomira.com), a 100 person company in Edmonton, Canada, is currently designing a glycopeptide-based vaccine for cancer. "It is more likely we can use it for colon cancer or breast

cancer," said Dr. R. Rao Koganty, PhD, vice president and general manager of the Synthetic Biologics Unit of Biomira. The work is still in preclinical stages, but Koganty says that the results are encouraging.

With the advent of procedures enabling efficient synthesis of oligosaccharides, the development of carbohydrate vaccines for recalcitrant infectious and parasite-borne diseases and various cancers appears increasingly feasible. "That is the vision … a wholly synthetic, wholly homogeneous vaccine," said Danishefsky. "If you have that vision, it has to be made by a total synthesis. The bad news is that even with the best methods, total synthesis is not a trivial undertaking. But the good news is that you don't need much compound. With a gram you could probably treat New York."

It is not such a stretch to imagine that with a few grams more, one could treat millions more around the world.

**Selected Reading**

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