Synthesizing mothers’ milk

Scientists are seeking ways to make beneficial but elusive sugars found in breast milk

by Tien Nguyen

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Steve Townsend’s daughter was only one day old when she rebuffed a nurse’s bottle of baby formula. Townsend’s wife, unable to nurse because her daughter was in the intensive care unit for some extra oxygen, was told to try pumping. To Townsend, a chemist on the cusp of a career at Vanderbilt University devoted to understanding and making molecules in breast milk, that moment was firsthand evidence of breast milk’s singular value.

Today’s infant formulas are much improved over formulas of decades past, but according to some scientists—and Townsend’s discerning daughter—they’re still a far cry from mothers’ milk.

Chemists hope to change that.

To create formula that more closely mimics breast milk, researchers are trying to make the elusive sugars that are abundant in breast milk, called human milk oligosaccharides. The molecules are thought to benefit babies’ health: They boost babies’ immune systems, block pathogens from sticking to cell surfaces, and promote healthy microbiomes by feeding good bacteria that can crowd out the bad. The sugars also have promising properties, such as antibacterial activity, that researchers are interested in exploring for uses beyond baby formula.

Infant formula that’s closer to breast milk could also benefit families when breastfeeding is not an option or their choice. Although breastfeeding has been championed in recent years, it’s not possible for all families, and the intense time commitment can make it difficult to

IN BRIEF

Mothers’ milk contains a gold mine of health-boosting molecules called human milk oligosaccharides. First discovered a century ago, these highly complex molecules are only beginning to be synthesized. To tackle this task, researchers are using a bevy of methods, from old-school synthesis to genetically engineered bacteria. Their efforts are already bearing fruit: Companies are adding oligosaccharides to formula, while new studies hint at broader benefits to come.

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keep up, particularly for working mothers. The American Academy of Pediatrics recommends that women breastfeed exclusively for the first six months, but on average, women in the U.S. receive six weeks of maternity leave or less, which Townsend says is a disgraceful dichotomy.

But getting a hold of the milk sugars hasn’t been easy. Mothers’ milk holds a mix of 200-plus oligosaccharides that can change from one day to the next. This constant flux of nutrients is good for babies because compositions can adapt to a nursing infant’s needs, but the variance is more of an inconvenience for researchers attempting to isolate the molecules. In the past decade, analytical chemists in the field have come a long way, identifying about three-quarters of the sugars’ structures.

Yet only a fraction of those compounds have been synthesized. That’s because bespoke sugar molecules must be strung together just so in chains that can be up to 30 sugars long. Meanwhile, the role of any individual oligosaccharide is largely unknown. Chemists are tackling the milk molecules’ synthesis from all angles, including automation, enzymes, brute synthetic force, and genetically engineered bacteria.

Even while researchers work out how to make more of the beneficial sugars, the baby food and infant formula market—worth $50 billion worldwide in 2017 and expected to shoot to $69 billion by 2023, according to Research & Markets—has started to incorporate the protective molecules into its products. So far, two simple human milk oligosaccharides that are abundant in breast milk have been approved and appear in products in the U.S. and Europe. And this month, three more oligosaccharides will be introduced into formula in a clinical trial in Germany.

**Construction ahead**

Scientists might be using different ways to put the puzzle pieces of human milk oligosaccharides together, but they all start with the same five basic sugars: glucose, galactose, fucose, N-acetylgalactosamine, and N-acetylmuramic acid.

For those, like Townsend, using traditional organic methods, the reactions to snap these sugars together are pretty straightforward. The rub is in linking them in the right order and orientation. Because each sugar contains multiple hydroxyl groups, making these connections requires chemists to plan lengthily, chesslike approaches; for each additional linkage, hydroxyl groups must be protected or deprotected to make sure only the correct hydroxyl group reacts.

Townsend’s synthesis of the four-sugar chain human milk oligosaccharide lacto-N-tetraose, for example, took about 20 steps (*Carbohydr. Res.* 2017, [DOI: 10.1016/j.carres.2017.02.001]). Now imagine trying to generate an oligosaccharide that is 20 or 30 sugars long.

Chemists like Nicola L. B. Pohl at Indiana University, Bloomington, are trying to make this process less painful—by programming a machine to do the work. Her lab has spent over a decade developing chemistry using an automated, liquid-handling system that can synthesize oligosaccharides in solution (*Org. Lett.* 2018, [DOI: 10.1021/acs.orglett.7b03940]).

The process relies on attaching fluorous tags to the starting sugar molecule. The tagged sugar gets coupled to another sugar, directly undergoes deprotection, and is purified by pulling out the product by the fluorous tag. The process can then be repeated to form longer oligosaccharides.
The slow step, which can take several hours, is evaporating away the solvent between reactions, Pohl says. But the major stumbling block, she says, is making the starting sugars that feed into the machine.

She points out that organic chemists can buy advanced intermediates from a treasure trove of molecular catalogs, but chemists dealing with carbohydrates have to start with basic sugars and then run six or more reactions to add the necessary protecting groups. “For us, we go back essentially to the natural product,” Pohl says.

Chemists in the carbohydrate community have started working together to overcome this issue. Eighteen research groups, including Pohl’s, were awarded U01 grants for collaborative research from the National Institutes of Health to work on the synthesis of carbohydrates. When the researchers realized their common frustration, seven of the groups decided on 28 modified sugars that would be useful in their research. Each lab agreed to make four reaction-ready sugars on a 10-to-12-g scale to be divvied up among the participating groups.

Pohl is also collaborating with researchers like Vy Dong at the University of California, Irvine, who’s working on developing catalysts that can differentiate between hydroxyl groups on the same sugar, which could ultimately reduce the number of protecting steps needed.

Another chemical automation group, led by Alexei V. Demchenko at the University of Missouri, St. Louis, uses repurposed high-throughput liquid chromatography instruments commonly found in labs (J. Org. Chem. 2016, DOI: 10.1021/acs.joc.6b01439). In one example, his lab was able to make a pentasaccharide compound—which takes weeks to prepare by traditional methods—in one day.

However, Demchenko says the future of oligosaccharide synthesis is likely to be enzymatic, a route that has the potential to make compounds faster and on a larger scale. “I’m a little jealous,” he says.

Labs that leverage enzymes have the advantage of very efficient syntheses. The challenge, however, is finding the right enzyme for the job, says Xi Chen, who has spent almost two decades doing just that.
Her University of California, Davis, lab has developed a chemoenzymatic method using multiple enzymes that carry out selective reactions and pump out human milk oligosaccharides on a gram scale (*Chem. Commun.* 2016, DOI: 10.1039/c5cc10646j). Her team is working to improve the technique’s efficiency so that the reaction runs to completion, allowing the researchers to simplify purification of the polar products, which is currently a bottleneck.

When it comes to industrial-scale synthesis of these compounds, companies have moved even further toward biological approaches. Recent patents filed for the two approved human milk oligosaccharides—2'-fucosyllactose (2'-FL), the most abundant oligosaccharide in breast milk, and lacto-N-neotetraose—show that manufacturers favor fermentation processes. This requires genetically engineering bacteria to produce the desired oligosaccharides. Developing the first products to contain human milk oligosaccharides took almost a decade (*Nutr. Rev.* 2016, DOI: 10.1093/nutrit/nwu025). That’s about the same amount of time it takes to come up with a new drug.

Chen says for larger oligosaccharides, fermentation processes may yield an undesirable product mixture and unwanted by-products. Although researchers would have a tough time coming up with synthetic and chemoenzymatic routes to make more complex oligosaccharides, she thinks it can be done.

Chemical strategies can still be important for human milk oligosaccharides because so few have been made, Pohl says. Her lab and others hope to make libraries of the natural oligosaccharides and analogs that could be valuable with just tens of compounds. Though analytic techniques in this area of research have matured, they still need pure standards for mass spectroscopy. “It’s hard to beat chemical synthesis to get diversity quickly,” Pohl says.

**Breast milk’s benefits**
As chemists continue to devise clever ways of making these complex compounds, their next challenge is unravelling the role and potential health benefits of individual oligosaccharides, some of which are already in baby formula.

Chen teamed up with Lars Bode, a biologist at the University of California, San Diego, and director of the Mother-Milk-Infant Center of Research Excellence, to evaluate the function of human milk oligosaccharides, as well as analogs, made using her method. They found that the natural milk oligosaccharide disialyllacto-N-tetraose protected newborn rats from necrotizing enterocolitis, a common and sometimes fatal intestinal disorder in preterm babies. Bode’s group recently observed the compound’s protective effect in a human cohort study as well (Gut 2017, DOI: 10.1136/gutjnl-2016-312819).

Bode’s lab found another promising effect: human milk sugars’ antibacterial activity against Group B Streptococcus, a result also reported by Vanderbilt’s Townsend. They observed that human milk oligosaccharides acted synergistically with existing antibiotics, and suggest that this could lower the amount of antibiotics required, which could help stave off antibacterial resistance.

Townsend’s lab has reported the oligosaccharides’ antibacterial activity against other strains of bacteria, and antibiofilm activity against a drug-resistant Staphylococcus aureus strain (ACS Infect. Dis. 2017, DOI: 10.1021/acsinfecdis.7b00183). As part of a department recognized for its ability to support drug development, he has plans to explore oligosaccharides as a possible antibiotic drug scaffold.

Both Bode and Townsend have unique access to oligosaccharides through their institutions’ human milk donation programs. Their labs have developed rigorous methods to selectively isolate oligosaccharides out of breast milk, which may contain hundreds or thousands of compounds, according to some estimates. “Our lab takes pride in our clean samples,” Bode says.

Human milk oligosaccharides are showing all sorts of interesting effects in the lab, Bode says, many of which his lab hopes to publish in the coming year.

Industry has been excited by the beneficial effects of human milk oligosaccharides as well.

Abbott added 2’-FL to its formula after a 2016 clinical trial showed that babies who were fed formula with the oligosaccharide had immune systems that were similar to those of breastfed babies (J. Nutr. 2016, DOI: 10.3945/jn.116.236919). The move, according to co-author Rachael Buck, a research fellow specializing in nutrition and immunology at Abbott, was “one of the biggest breakthroughs in infant formula in decades.”

Another clinical trial, by Jennewein Biotechnologie in Germany, will soon be testing a formula with a blend of five abundant human milk oligosaccharides: 2’-FL and four others produced via a proprietary fermentation process.

The trial will enroll 300 babies (less than 13 days old). A third will receive standard formula, another third will get standard formula with the five oligosaccharides added, and the remainder will be exclusively breastfed. Clinicians will collect breast milk and fecal samples and track any effects on the babies’ microbiomes.

Katja Parschat, Jennewein’s head of research and development who’s running the trial, says the company hopes to get approval for the new oligosaccharides by U.S. and European regulatory boards. It took several years in Europe and about a year and a half in the U.S. for the first oligosaccharides to gain approval.
Some people, however, question the wisdom of adding the human milk oligosaccharides the way they’re currently regulated. In the U.S. and Europe, the concentration of human milk oligosaccharides in formula is limited to about 2 g/L, far less than the 20–25 g/L typically present in breast milk.

“It’s not clear if adding that amount of oligosaccharide will be enough,” says Bethany M. Henrick, director of immunology and diagnostics at Evolve BioSystems.

But her bigger concern is whether babies have the proper gut bacteria needed to break down the oligosaccharides. “You can’t have one without the other,” she says.

Evolve BioSystems offers a product called Evivo that contains a strain of the bacteria known to consume oligosaccharides, *Blidobacterium infantis* EVC001. A study of 66 infants conducted by Evolve shows that its probiotic can persist in babies’ microbiomes, which the company suggests leads to healthier guts (*mSphere* 2017, DOI: 10.1128/mSphere.00501-17). Recently, Evolve received funding from the Bill & Melinda Gates Foundation for a clinical trial in Bangladesh to treat malnourished children with Evivo.

Henrick also worries about how a baby’s microbial community will be affected by consuming only a few oligosaccharides rather than the incredibly complicated mix found in breast milk. It’s something UCSD’s Bode wonders about too. “Maybe we’ll find out in about 20–30 years,” he says.

Until then, scientists expect to know a lot more about the role of individual molecules in human milk, Townsend says, “It’s a really exciting area,” he says. “Within the next five to 10 years we should be able to identify the sugars’ biological targets and start to figure out exactly how mom is able to protect baby.”

Soon, Townsend will have even more motivation at home to make progress in the lab. His wife, LaToya Townsend, is pregnant with their second child. She breastfed their first daughter, who’s now a precocious toddler, exclusively for 12 months while finishing graduate school and relocating to Nashville. Now a full-time social worker, she’ll get four months of paid maternity leave before joining the legions of women juggling pumping at work.

Whether she decides to donate any of her breast milk to Vanderbilt—possibly being the latest contributor to Townsend’s research—remains to be seen.

CORRECTION: This article was updated on July 12, 2018, to reflect that the human milk oligosaccharides to be tested in Jennewein’s clinical trial will include one of the approved oligosaccharides, not two.
COMMENTS

Sherri Mason  
(Fri Jul 06 08:43:48 EDT 2018)  
This article comes to me by way of my spouse, who is the engineer, and I find it incredibly fascinating! As a healthcare consultant, and a long-time nurse and mother of a preemie now 15 years old, I find this research and progress so encouraging. The direct result is of course to benefit the health of our tiniest babies and those where the mother cannot provide breast milk, but the avoidance of infection through protection against Strep B and reduced lengths of stays for these babies will logically reduce healthcare costs and improve maternal-infant and family bonding.  
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