

Breast milk supplies more than just nutrition for babies.



## Nature's first functional food

Breast milk feeds helpful microbes, fights harmful ones, provides immunity, and jump-starts a newborn's life

By Trisha Gura

**B**lood, urine, saliva, and spinal fluid. Those are the human bodily fluids most explored by scientists over the decades. Yet any woman who has ever nursed a newborn will cite a major omission: breast milk. Researchers long ago unraveled the basics of this maternal liquid. But until recently, few have given it serious attention with sophisticated analytical techniques. Breast milk was “ignored as not modern,” for the last half-century, says epidemiologist Ardythe Morrow of Cincinnati Children's Hospital Medical Center in Ohio. But now, it's “an exploding area of science.”

The resurgence has its origins in a long-

standing conundrum: Breast milk abounds with complex carbohydrates called oligosaccharides that humans can't digest but beneficial bacteria can thrive on. Fifty years ago, when the oligosaccharides were discovered, investigators lacked the technology to deduce their structure and determine their effect on what is now called the infant gut microbiome (the myriad bacteria that naturally reside in human intestines, beginning at birth). Unable to progress significantly, scientists lost interest in milk-microbe connections.

Now, thanks to breakthroughs in analytical chemistry and a growing interest in the microbial fauna in the human body, as well as a movement touting the benefits of breast-feeding, those connections are be-

ing explored once again. Some researchers have focused on making better use of the microbiome fostered by milk, while others have documented how breast milk does more than feed a newborn and its “good” bacteria. Mother's milk also contains an evolving stockpile of compounds that thwart pathogens, foster a robust immune system, and perform other functions. Most recently, researchers have discovered that mom provides inactive enzymes in her milk that turn on in the infant gut and clip out bioactive molecules from other milk proteins. “Milk is really a genius fluid that was outrageously understudied,” says microbial ecologist David Mills of the University of California (UC), Davis. “If we can identify components of human breast milk that are important, then we can understand the wisdom of milk—and take advantage of them.”

**AT FIRST BLUSH, BREAST MILK** is a buffet of fats, proteins, and sugars, in a ratio of about 1-to-3-to-7. Until recently, scientists viewed the fluid mainly as food for a rap-

idly growing newborn. Yet, mother's milk also teems with immune cells, such as macrophages, stem cells for regeneration, and hundreds to thousands of bioactive molecules—some fatlike, others protein in nature, and still others that are indigestible oligosaccharides. These bioactive molecules can protect against infection, ward off inflammation, spur immune system and organ development—and, in the case of oligosaccharides, shape the infant microbiome, scientists have found. Milk “is not primarily about nutrition,” Morrow says. “Rather [it's about] immune protection.”

Signs of that protection began to emerge almost 130 years ago with the discovery that breast-fed infants survived more often than bottle-fed ones. In searching for reasons why, pediatricians and microbiologists analyzed the bacterial composition of the feces of both types of infants, which reflects their gut microbiome, and found significant differences. Meanwhile, chemists at the turn of the 20th century noted that human breast milk contained “a different type of lactose” than cow's milk did. Then, in the 1950s, Nobel Prize winner Richard Kuhn and physician Paul György showed that this “different type” consisted of hundreds of oligosaccharides that promoted the growth of microbes called bifidobacteria. But the work paused, as chemists spent the next 50 years advancing techniques necessary to sort out the complexity of the oligosaccharides.

In 2006, a team at UC Davis led by food chemist Bruce German and his graduate student Robert Ward helped reignite interest in the milk-microbiome connection. The group enlisted analytical chemist Carlito Lebrilla, who used mass spectrometry and a sophisticated chromatographic technique to characterize the oligosaccharide repertoire. Lebrilla identified nearly 200 unique human milk oligosaccharides (HMOs) and handed the mix to Mills, who, along with German, assumed a plethora of bacterial species would feast on the sugary cocktail.

“But they didn't.” German says. “It was a jaw-smacking moment.”

Only one type grew robustly: *Bifidobacterium longum biovar infantis* (*B. longum* *bv. infantis*). Scientists are not yet certain exactly how the bacteria first enters the infant's intestinal tract—possibly when a fetus begins swallowing amniotic fluid and later as the infant passes through the vaginal tract. Breast milk, which contains small numbers of the bacteria, is another possible route. Assisted by the HMOs in breast milk, the organism eventually multi-

plies to dominate the breast-fed baby's microbiome, making up as much as 90% of it. After weaning, it eventually drops to 3% of the adult microbiome. The UC Davis team wondered: Why did *this* organism so rule the budding infant microbiome?

A year later, Mills came up with an answer. After sequencing the genome of *B. longum* *bv. infantis*, he learned that it carries the precise genes for all the enzymes needed to digest the milk's oligosaccharides—other bacteria, even closely related ones, don't share all of those genes. “Clearly this bacterium coevolved” with humans, German says.

Why would a new mother expend energy producing food for an organism other than

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David Mills, UC Davis

her offspring? German says it's pretty clear. “Mother is recruiting another life form to babysit her baby.”

By babysitting, German refers to the growing list of protective functions in a newborn that *B. longum* *bv. infantis* appears to orchestrate. For example, the bacterium consumes the oligosaccharides whole before other nasty microbes can digest them, starving out the bad bugs. *B. longum* *bv. infantis* also produces molecules called short-chain fatty acids that serve as favorite food for other beneficial bacteria and help guide the cells that line the infant intestine in how to use energy and mount an immune defense.

Apart from promoting good bacteria, human milk oligosaccharides themselves can ward off dangerous bacteria such as *Salmonella*, *Listeria*, and *Campylobacter*, causes of diarrhea and infant mortality. The HMOs closely mimic carbohydrate structures on the infant's gut cells. Because many pathogens typically attach to these cell-bound structures to infiltrate the gut, mother's milk, in essence, floods the infant's gastrointestinal tract with decoys that bind the pathogens and keep them off the intestinal wall.

**NEONATOLOGISTS ARE NOW HOPING** to turn the growing understanding of *B. longum* *bv. infantis* to their advantage, most immediately by ameliorating, or pre-

venting, an often deadly disease called necrotizing enterocolitis (NEC) that tends to develop in premature infants. Starting as a serious bacterial infection, NEC overwhelms the gut several weeks after birth. The intestinal tissue dies; surgeons try to remove it; and the baby often suffers severe and permanent disability, neurological damage, and—in 20% to 30% of cases—death.

NEC can strike just when it seems a premature infant is out of the initial dangers of being born too early. “I can think of a baby,” says neonatologist Mark Underwood of UC Davis, “who one day was looking good and on the next day was pooping blood. By that night was dead. You see that in a kid who you thought was past the worst of it, and it is just so devastating.”

Theories abound to explain the condition: Premature infants have a leaky gut that lets bacteria through, or cells lining their intestine exhibit an overreactive inflammatory response. Premature infants may also not get enough breast milk: Studies show that the risk of NEC plummets six- to 10-fold if preemies are given breast milk rather than formula.

Yet, even when mother's milk is provided in a feeding tube, NEC can still take hold. The problem may not be a lack of milk, but rather a deficient microbiome—one lacking enough *B. longum* *bv. infantis*, for example. Simply, premature infants carry premature intestinal fauna, Underwood and others say.

In some studies, attempts to speed the maturation of the microbiome, just as physicians give substances to preemies to promote lung development, have shown little success. In one recent effort involving preterm infants, the UC Davis team tried out different combinations of formula and breast milk with and without the right oligosaccharides. The researchers discovered that none of the interventions changed the microbiota of the preemies. By contrast, some studies in full-term infants indicate that adding HMOs to formula or breast milk promotes beneficial microbes. So why are preemies not responding similarly?

“It's a different environment that premature infants live in,” notes neonatologist Barbara Warner of Washington University School of Medicine in St. Louis. Catheters, breathing tubes, and other instrumentation breed infections by microbes not normally in an infant. Also, preemies get antibiotics for long periods of time, wiping out beneficial bacteria.



**DESPITE MIXED RESULTS**, clinicians trying to help premature infants and other newborns now view mother's milk and its promotion of a good microbiome as so key that many are developing and testing supplements containing probiotics (the bacteria) and prebiotics—food that will stimulate the beneficial bacterial growth. Clinical researchers in Europe, Canada, and elsewhere have completed nearly 40 studies using pre- or probiotics, some containing *B. longum* bv. *infantis*, in premature infants, estimates Allan Walker of Boston's Massachusetts General Hospital and Harvard School of Public Health. The most recent, published online in January in *The Journal of Pediatrics*, showed that a probiotic product that contains *B. longum* bv. *infantis*, one readily available in North America, substantially reduced the frequency of NEC in a Montreal, Canada, neonatal intensive care unit.

Few such studies have been done in the United States, however. Although for adults probiotics are considered supplements and go unregulated, introducing microbes into premature infants falls under the same U.S. Food and Drug Administration regulations as vaccines or new drugs. Anticipating increased development costs and time, U.S. researchers have either pursued other strategies or partnered with collaborators elsewhere.

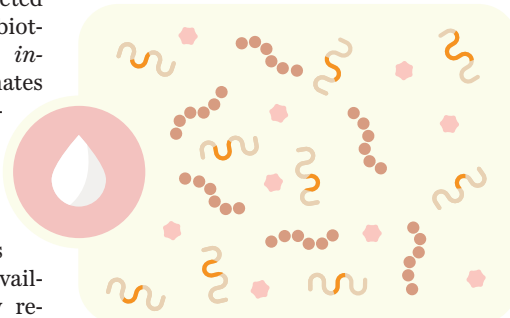
For example, Walker's group is working with neonatologists in Chile to test a specific sugar-containing molecule, or glycan, found in mother's milk. Studies in newborn mice, as well as in a tissue model of the human premature gut, show that the glycan damps down proinflammatory factors, while boosting anti-inflammatory ones. Chilean researchers will supplement mother's breast milk with the glycan and look for a reduction in the incidence of infection and death in premature infants.

While also pursuing the idea of introducing *B. longum* bv. *infantis* or its food in preemies, the UC Davis researchers have gone back to basic studies of mother's milk for further inspiration. They began by analyzing the fluids taken from the gastric tubes of premature infants after feeding. To their surprise, the researchers learned that the infant stomach is not wildly acidic and stocked with aggressive enzymes, as previously thought. The group reported online in April in *The Journal of Nutrition* that the infant stomach seems geared to digest very few proteins, mainly  $\beta$ -casein, but also lactoferrin, lysozyme, and  $\alpha$ -lactalbumin. All are found in breast milk. The enzymes that were present were not chopping up proteins randomly,

## Breast milk's hidden gifts

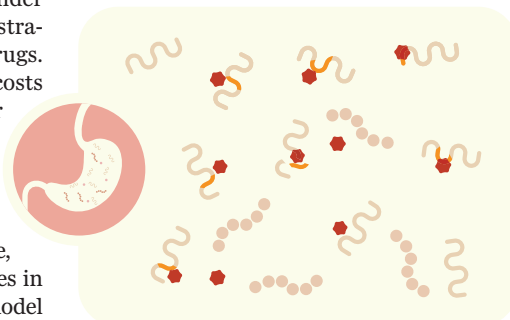
A complex mixture of proteins, fats, and sugars, mother's milk does far more than nourish the growth of a newborn. Some of its components enlist helpful bacteria.

### In the milk



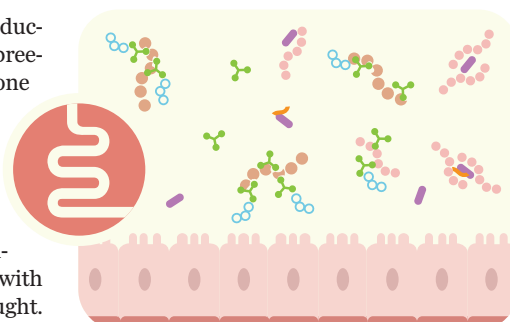
Milk transfers complex carbohydrates called oligosaccharides (●●●), proteins (〰), and inactive enzymes (●) to the baby.

### In the stomach



Activated enzymes (●) cut useful peptides (〰) from proteins. The peptides aid the immune system.

### In the gut



Good bacteria (●●) thrive on oligosaccharides, which, along with peptides, also mop up bad bacteria (●) to keep the gut healthy. The good bacteria make beneficial fatty acids (〰).

Illustration: G. Grullón/Science

but rather were clipping them at very specific sites in their sequence, generating 603 peptides. Some of these are known to inhibit the growth of pathogenic bacteria or regulate the immune system.

What's more, the UC Davis team recently discovered that some of the digestive enzymes they found in infants' guts after feeding are actually produced by the mother and delivered, inactive, in her milk. Something in the infant's stomach later turns them on so they can free up the active peptides from the full milk proteins. The UC Davis team theorizes that supplementing milk with the latent enzymes will promote the formation of the beneficial peptides in premature infants.

Industry is watching this and the oligosaccharide developments closely. "It is a rat race," says nutritional scientist Lars Bode of UC San Diego. "Whatever formula company comes out with the first oligosaccharide to add to their product, that will make a huge shift in the market. Everyone is working on it."

For example, German, Mills, and the UC Davis team started a company called Evolve Biosystems Inc. to advance their own combination of prebiotics and probiotics. Because scientists cannot get enough donor human milk to isolate enough prebiotics, German's group is working instead with bovine milk, which contains small amounts of HMOs. Infants have been drinking dairy-based formulas for decades—without apparent harm—the researchers reason. Hence, the approach should get around some regulatory hurdles.

But the sugars in cow's milk often differ substantially in their structure and abundance from those in human breast milk. Thus, investigators such as biochemist David Newburg of Boston College have set out to synthesize HMOs. In 2004, Newburg showed that an oligosaccharide found in breast milk called 2'-fucosyllactose is effective in warding off the pathogenic bacteria that cause cholera, a diarrheal disease. With his own money, he co-founded Glycosyn LLC to test the oligosaccharide and its derivatives. The company makes them by inserting the genes for various sugar-building enzymes into yeast and bacteria. Newburg predicts he is about a year away from marketing a nutritional supplement.

This intense rush of research and commercial development only highlights the remarkable complexity and powers of the milk every mom naturally makes for her newborn. "Evolution has delivered the functional food," Mills says. "We just need to understand it." ■

Trisha Gura is a science writer in Boston.