

PHYSIOLOGY

# Understanding the mother-breastmilk-infant "triad"

## Breastmilk research holds important opportunities to improve maternal-child health

By **Lars Bode[1], Arjun S. Raman[2], Simon H. Murch[3], Nigel C. Rollins[4], Jeffrey I. Gordon[2]**

Breastfeeding and breastmilk exert remarkable influence on infant survival and health (*1*, *2*), including reduced risk from infections and promoting various aspects of postnatal development. The many maternal benefits include protection from breast and ovarian cancer and cardiometabolic disorders. Although the mechanisms underlying some of these benefits have been elucidated, the origins of others that have been reported, such as influence on adult IQ and later protection against obesity and diabetes, remain more obscure. Hence, timely investments in research designed to clarify the operations and biological effects of the mother-breastmilk-infant "triad," and their translation into public health, are needed.

Breastmilk does not stand alone; maternal physiology, breastmilk composition, and infant physiology are parts of a coadapting system, with variations in each influencing the trajectory of infant development and maternal health. In addition to macronutrients and micronutrients essential for child survival, breastmilk contains other myriad bioactive components, including cells and microbes (*3*, *4*). Breastmilk can be considered a "live tissue" whose composition varies between women and changes over the course of lactation. Structurally diverse human milk oligosaccharides (HMOs) represent the third most abundant nonaqueous component of breastmilk (after lactose and lipids). One

[1]Department of Pediatrics and Larsson-Rosenquist Foundation, Mother-Milk-Infant Center of Research Excellence, University of California, San Diego, La Jolla, CA, USA. [2]The Edison Family Center for Genome Sciences and Systems Biology, and the Center for Gut Microbiome and Nutrition Research, Washington University School of Medicine, St. Louis, MO, USA. [3]Warwick Medical School, University of Warwick, Coventry, UK. [4]Departments of Maternal, Newborn, Child, and Adolescent Health and Ageing, World Health Organization, Geneva, Switzerland. Email: lbode@ucsd.edu, jgordon@wustl.edu

A Rohingya Muslim refugee holds her malnourished child in Bangladesh. Better understanding of breastmilk could improve therapeutic foods to treat undernutrition.

prominent example illustrating how maternal genotype affects breastmilk composition is a single-nucleotide polymorphism that introduces a premature stop codon in the fucosyltransferase-2 (*FUT2*) gene. This mutation abolishes the ability to synthesize α(1-2)-fucosylated HMOs. The presence or absence of these HMOs creates specific maternal "lactotypes," known as secretors and nonsecretors, respectively, with the breastmilk of secretors conveying reduced risk of common forms of infectious diarrhea (*5*).

Comprehensive characterization of the components of each axis of the triad through longitudinal and cross-sectional studies of maternal-infant cohorts has expanded markedly. Increasingly, high-throughput analytical methods have been used to characterize more than 150 different HMO structures, and intra- and interpersonal variations in their representation within and across different populations (*6*). Other components of breastmilk, including compounds associated with the membrane that surrounds milk fat globules, microRNAs, and bacterial constituents, as well as antibodies and immune cells, are being actively cataloged and characterized. In addition to quantifying the products of metabolism in infants and their mothers by mass spectrometry, platforms are now available for simultaneously measuring the concentration of thousands of proteins circulating in blood that are biomarkers and regulators of numerous physiologic, metabolic, and immune processes, as well as other facets of growth and homeostasis (*7*). Furthermore, recent studies have highlighted how, with the use of culture-independent methods, features of gut microbial community development in infants and young children can be used as a readout for their nutritional status (*8*, *9*).

Although datasets pertaining to each axis are available, considerably more work is needed to quantitatively relate how environmental influences affect the triad and in turn, how variations in each of its axes influence the other (see the figure). For example, it will be interesting to discover what other maternal genetic factors affect biosynthesis of HMOs and other milk constituents. The mechanisms that link maternal nutritional status and other aspects of their physiology to breastmilk features and infant growth phenotypes are also an important issue. Additionally, which signaling pathways allow infant health status to regulate maternal biology, including breastmilk composition, should be investigated. How varied sociocultural, behavioral, and environmental factors shape and perturb the development of the triad is important to understand so that a "normal" range can be defined. Two disorders, childhood malnutrition and necrotizing enterocolitis (NEC), illustrate how a deeper understanding of the mother-breastmilk-infant triad could improve child health with potential lifelong benefits, and how some of the analytic challenges might be surmounted.

Childhood malnutrition contributes to 45% of deaths worldwide in those under the age of five; it manifests early in life and involves disruption of multiple biological systems fundamental to healthy growth, including host pathways influenced by the developing gut microbiota, which are key consumers of breastmilk constituents (*8*, *9*). One approach for obtaining new insights about disease pathogenesis is to conduct longitudinal studies of healthy and malnourished children living in areas where disease burden is high, and to comprehensively characterize the plasma proteomes, metabolomes, and developing microbial communities of malnourished infants and their healthy counterparts, their mothers' breastmilk composition, and the products of microbial HMO utilization.

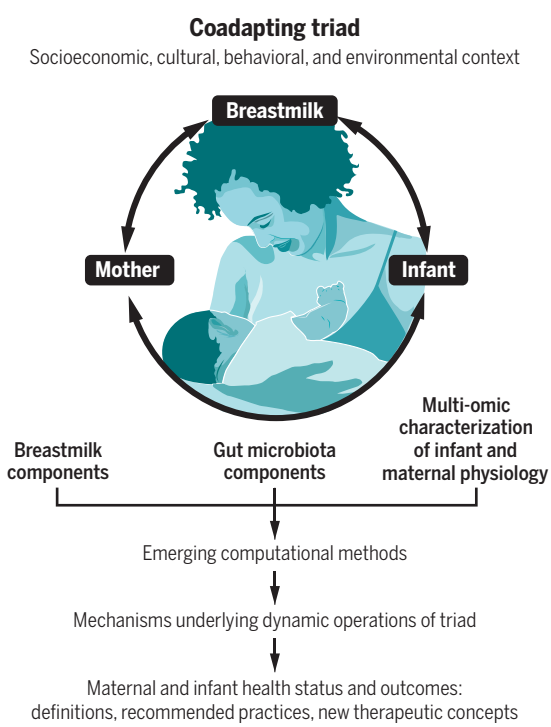
A strategy for defining functionally important interactions between triad components is to borrow from studies conducted in disparate fields where statistical covariation is used for "feature reduction." Components that covary with each other are deemed important for defining the behaviors or functions of dynamic complex systems. Applying this approach to the developing gut microbiota of healthy members of a Bangladeshi birth cohort sampled monthly from 1 to 60 months of age disclosed a network composed of 15 covarying bacterial taxa (*9*). The abundances of these taxa describe normal gut microbial community assembly in healthy members of birth cohorts residing in diverse geographic locales and are useful for quantifying the degree of impaired microbial community development in children with moderate and severe acute malnutrition.

From the limited evidence available, microbiota immaturity associated with these conditions is not repaired with standard therapeutic foods. Affordable, culturally acceptable complementary foods have been identified that in combination repair the gut microbiota of Bangladeshi children with moderate acute malnutrition toward a state resembling that of age-matched, healthy growing children. This is accompanied by increases in numerous blood plasma protein biomarkers and mediators of growth, bone formation, neurodevelopment, metabolism, and immune function (*8*, *9*).

These findings support the idea that healthy growth is linked in part to healthy development of the gut microbiota. They also raise the question of what factors shape microbial community development during the period of exclusive breastmilk feeding, and as children transition to complementary foods during the weaning period. Members of the bacterial genus *Bifidobacterium*, notably *B. longum* subsp. *infantis*, have suites of genes involved in the import and metabolism of HMOs. It is important that efforts be directed to defining the representation of *B. infantis* and other HMO-consuming bacteria in healthy versus malnourished in-

## How does breastmilk affect maternal and infant health?

Mechanistic insights hold the promise of providing more informative definitions of health status, better predictions of health outcomes, improved recommendations for preventing disease, and new therapeutic targets.




GRAPHIC: N. CARY/*SCIENCE*

fants and their mothers. This information, together with characterizing the representation of genes involved in HMO acquisition and degradation in different bacterial strains cultured from these children, would allow an assessment of (i) whether and how the presence of these different organisms and their genome features correlate with maternal breastmilk composition and (ii) the degree to which products of breastmilk metabolism correlate with host features. The answers, from analyses of human biospecimens as well as animal models colonized with consortia of human gut microbes representing different stages of community assembly (*10*, *11*), could have important therapeutic implications. These include the development of new probiotic, HMO-based prebiotic and/or synbiotic (prebiotic combined with probiotic) therapies (*12*).

NEC provides a different type of opportunity to characterize the mother-breastmilk-infant triad. One of the most common and fatal gastrointestinal disorders in preterm infants, NEC develops within the first few weeks of delivery. It is characterized by destruction of the integrity of the intestinal wall, invasion of luminal bacteria, marked inflammation, and sepsis. Maternal and infant physiology are immature after preterm delivery in terms of producing and digesting breastmilk. Moreover, the use of antibiotics and other medications and interventions, when both mother and infant face serious and often life-threatening crises, further disrupts the mother-breastmilk-infant triad, including initial colonization of the infant intestine. Although breastmilk composition is not fully adapted to the physiological needs of the premature infant, breastmilk feeding, compared to enteral feeding with specialized breastmilk substitutes, reduces NEC incidence by 6- to 10-fold (*13*). The mechanisms underlying these protective effects remain largely uncharacterized.

HMOs significantly improve survival and reduce pathology in a neonatal rat model of NEC, leading to the identification of the HMO, disialyllacto-*N*-tetraose (DSLNT), as a protective factor (*14*), likely through its direct interactions with gut epithelial and immune cells. A multicenter study of mothers and their very-low-birthweight infants found that infants who developed NEC received breastmilk containing less DSLNT than infants who did not develop NEC (*15*). Proof of a causal relationship requires a randomized controlled clinical trial, which raises several challenges, including the availability of DSLNT and ethical considerations if control groups of high-risk infants were to be treated with formula alone. More generally, NEC illustrates the need to comprehensively define states of "triad immaturity." This would entail longitudinal studies of the set of features that define breastmilk given to prematurely born neonates who do and do not develop this devastating disease. It would also require a simultaneous effort to obtain comprehensive definitions of the biological characteristics of chronologically age-matched preterm infants with and without NEC, as well as of their mothers.

Mothers face a "balancing-act" between various socioeconomic, cultural, and even marketing pressures to maintain or forego breastfeeding and their motivation to provide their infants with what is best for their health and development. This balancing act is perpetuated in part by confusion surrounding the respective attributes of breastmilk versus breastmilk substitutes, with consumer understanding being heavily influenced by commercial interests. Aspirational goals include new parameters for defining health status and deeper understanding of how health outcomes are related to breastfeeding and breastmilk components. Within a risk-stratified continuum of care, knowledge of the latter has potential therapeutic implications and opportunities, personalized to the circumstances of an individual mother and her infant (*1*). Such efforts will not only provide new appreciation of the remarkable properties of nature's first food, but also serve to further develop analytic approaches that yield insights into the dynamic systems that direct infant development. ■

## REFERENCES AND NOTES


1. C. G. Victora *et al.*, *Lancet* **387**, 475 (2016).
2. N. C. Rollins *et al.*, *Lancet* **387**, 491 (2016).
3. M. Witkowska-Zimny, E. Kaminska-El-Hassan, *Cell. Mol. Biol. Lett.* **22**, 11 (2017).
4. A. Boix-Amorós, M. C. Collado, A. Mira, *Front. Microbiol.* **7**, 492 (2016).
5. L. Mottram, G. Wiklund, G. Larson, F. Qadri, A.-M. Svennerholm, *Sci. Rep.* **7**, 10649 (2017).
6. L. Bode, *Glycobiology* **22**, 1147 (2012).
7. B. Lollo, F. Steele, L. Gold, *Proteomics* **14**, 638 (2014).
8. J. L. Gehrig *et al.*, *Science* **365**, eaau4732 (2019).
9. A. S. Raman *et al.*, *Science* **365**, eaau4735 (2019).
10. C. A. Cowardin *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **116**, 11988 (2019).
11. L. Feng *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **117**, 2622 (2020).
12. B. M. Henrick *et al.*, *Pediatr. Res.* **86**, 749 (2019).
13. J. Meinzen-Derr *et al.*, *J. Perinatol.* **29**, 57 (2009).
14. E. Jantscher-Krenn *et al.*, *Gut* **61**, 1417 (2012).
15. C. A. Autran *et al.*, *Gut* **67**, 1064 (2018).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IMMUNOLOGY

# Origins of peanut allergy-causing antibodies

## Analysis of gut-produced antibodies raises questions about how food allergy arises

*By* **Duane R. Wesemann**[1] *and* **Cathryn R. Nagler**[2,3]

Some people produce immunoglobulin E (IgE) antibodies to proteins in common foods. As a result, these foods can trigger severe allergic inflammation (anaphylaxis). There are several structurally and functionally distinct antibody isotypes (IgM, IgD, IgG, IgA, and IgE), and which isotype binds to a target molecule (antigen) influences what happens next. For example, IgG that binds peanut proteins is harmless, but IgE bound to the same proteins can induce anaphylaxis and death. Therefore, how, where, and why allergen-reactive IgE is made are decades-old questions. Hoh *et al.* (*1*) found that gut tissue is a likely place for IgE development in peanut-allergic individuals. In addition, despite vast sequence possibilities, they found that many individuals share similar peanut-reactive IgE DNA sequences. This suggests that IgE antibodies in different individuals recognize peanut proteins in a similar manner, which could inform strategies for pharmacological interventions.

Antibodies are produced by cells of the B lymphocyte lineage and consist of four Ig polypeptide chains—two identical heavy (H) chains and two identical light (L) chains—and each chain has a variable (V) region and a constant (C) region. The V region forms the surface that physically binds to antigens such as peanut proteins. The C region of IgH (CH) dictates antibody

[1]Department of Medicine, Division of Allergy and Clinical Immunology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA. [2]Department of Pathology, Biological Sciences Division, The University of Chicago, Chicago, IL, USA. [3]Pritzker School of Molecular Engineering, The University of Chicago, Chicago, IL, USA. Email: dwesemann@bwh.harvard.edu; cnagler@bsd.uchicago.edu

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