Power of Cognition and Growth: Nutritional impact of illness to brain and Growth development

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Breast milk is the best for babies

- **The World Health Organization recommends exclusive breastfeeding for the first six months of life. Unnecessary introduction of bottle feeding or other food and drinks will have a negative impact on breastfeeding.**

- **After six months of age, infants should receive age-appropriate foods while breastfeeding continues for up to two years of age or beyond.**

- **Consult your doctor before deciding to use infant formula or if you have difficulty breastfeeding.**

Source: https://www.healthhub.sg/live-healthy/530/SIFECS%20Code%20of%20Ethics

Breastfeeding is best

- **Breastfed babies fall sick less often**
  - **Less diarrhea**
  - **Less ear infections**
  - **Less respiratory infections**

Duijts L, Jaddoe VW, Hofman A, Moll HA. Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. Pediatrics. 2010 Jul;126(1):e18-25.

Breast Milk is Unique!

Anti-microbial Factors
- Secretory IgA, IgM, IgG
- Lactoferrin
- Lysozyme
- Complement C3
- Leukocytes
- Bifidus factor
- Antiviral mucins, GAGs
- Oligosaccharides

Growth Factors
- Epidermal (EGF)
- Nerve (NGF)
- Insulin-like (IGF)
- Transforming (TGF)
- Polyamines

Hormones
- Feedback inhibitor of lactation (FIL)
- Insulin
- Prolactin
- Thyroid hormones
- Corticosteroids, ACTH
- Oxytocin
- Calcitonin
- Parathyroid hormone
- Erythropoietin

Transporters
- Lactoferrin (Fe)
- Folate binder
- Cobalamin binder
- IgF binder
- Thyroxine binder
- Corticosteroid binder

Digestive Enzymes
- Amylase
- Bile acid-stimulating esterase
- Bile-stimulating lipases
- Lipoprotein lipase
- Ribonuclease

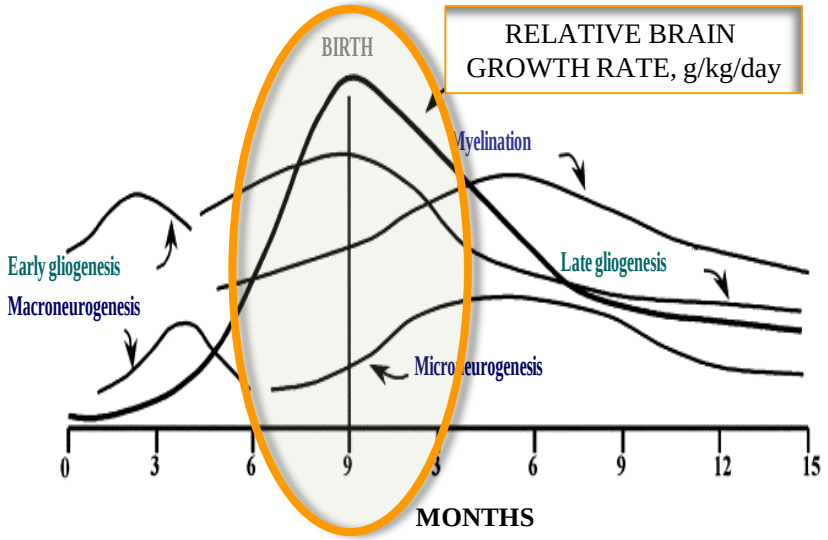
Cytokines and Anti-Inflammatory Factors
- Tumor necrosis factor
- Interleukins
- Interferon
- Prostaglandins
- a1-anti-chymotrypsin
- a1-anti-trypsin
- Platelet-activating factor: acetyl hydrolase

Human Milk Oligosaccharides
- 2’-FL
- 3-FL
- 3’-SL
- 6’-SL
- DFLac
- LNnT
- LNT
- Other HMOs

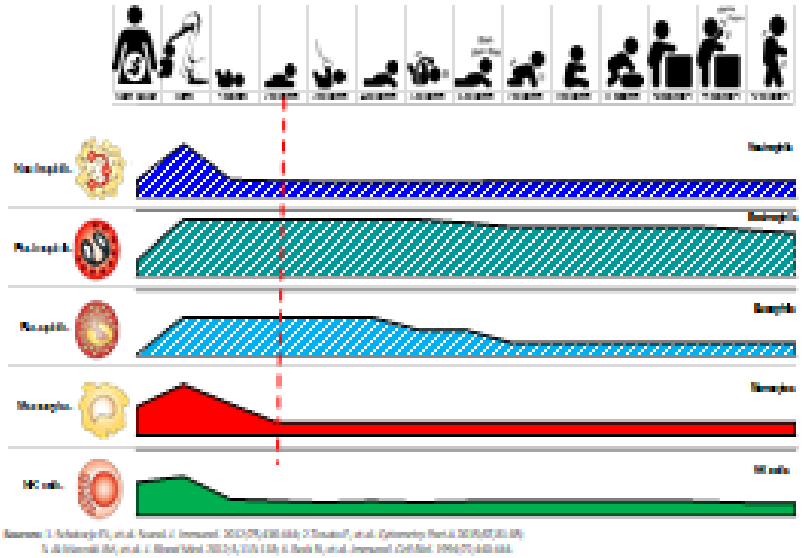
Others
- Casomorphins
- d-sleep peptides
- DNA, RNA
- Long chain polyunsaturated fatty acids (LCP)
- Carotenoids
- Nucleotides

First year is the golden window for both brain and immunity development

Rapid brain development

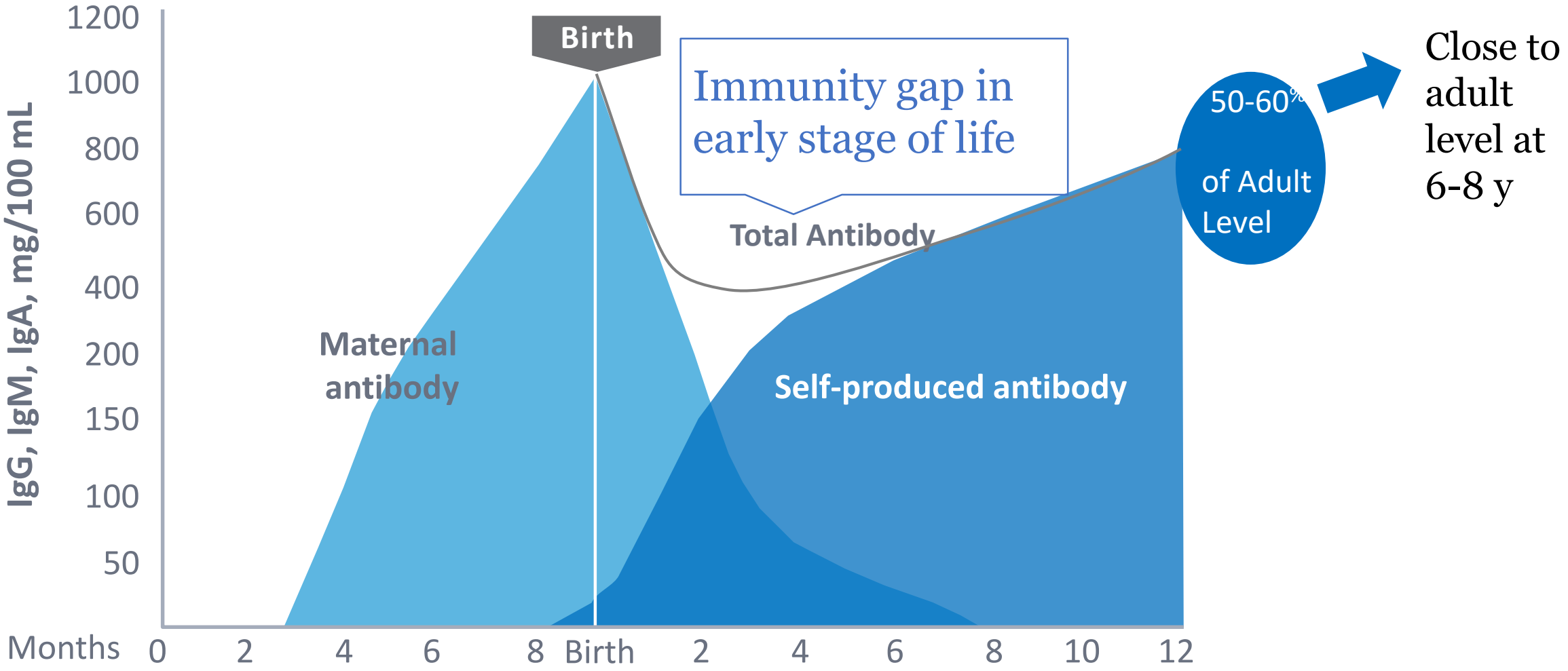


Innate Immune Cells Reach Adult Levels by 12 Months



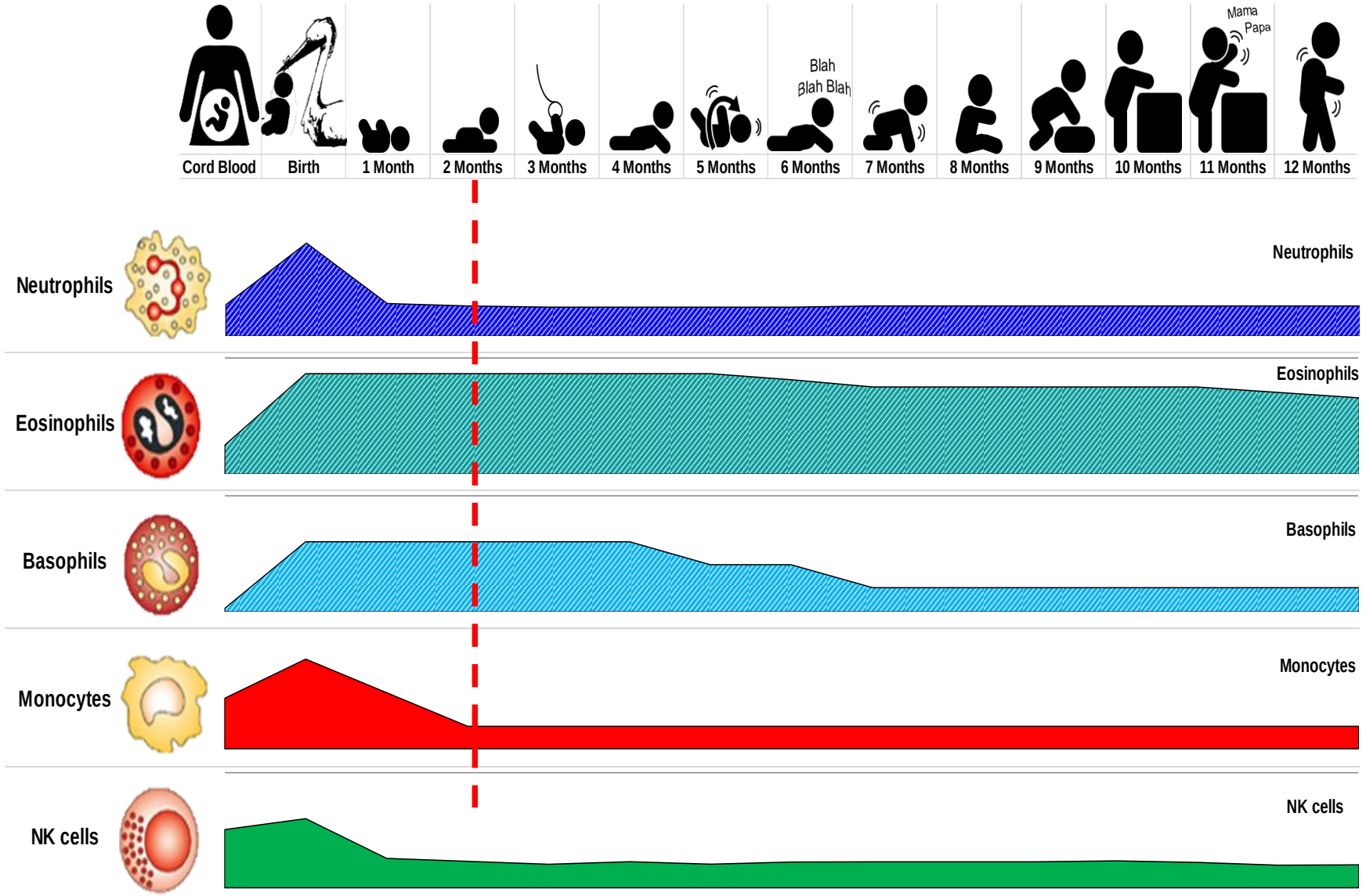
This critical window happens once in a lifetime.
Once it closes, it does not re-open.
Early intervention is key.

Immunity Gap in Early Stage of Life



Stiehm, Immu disorders in infants and children, 3rd ed. 1989;
Male, Immunology. 2013

Innate Immune Cells Reach Adult Levels by 12 Months



Schatorje EJ, et al. Scand. *J. Immunol.* 2012;75,436-444; Tosato F, et al. *Cytometry Part A* 2015;87,81-85; Al-Marzoki JM, et al. *J. Blood Med*. 2012;3,113-118; Beck R, et al. *Immunol. Cell Biol*. 1994;72,440-444.

Newborn infants are at high risk of infection due to naivety of the immune system

- No memory B cells, slow antibody production
- Antibody production to polysaccharide-encapsulated bacteria is particularly impaired (group B strep and pneumococci)
- T cell-mediated immunity is naïve
- Inefficient anti-viral responses lead to high morbidity with EBV, RSV, and enteroviruses

Kerperien J, Schouten B, Boehm G, et al. *Recent Advances in immunology to target cancer, inflammation, and infections*: InTech Open; 2012:315–334.; Niers L et al. *Nutr Rev*. 2007;65(8 Pt 1):347–60.; Vighi G, Marcucci F, Sensi L. *J. Clin Exp Immunol*. 2008;153 Suppl 1:3–6.

EBV – Epstein-Barr Virus

RSV – Respiratory syncytial virus

First Year Is an Important Window for Innate and Adaptive Immune System Development

Innate immunity is the first line of defense[1]

- Immediate response
- Induces adaptive immunity

Integrates with adaptive immunity

Adaptive immunity is the second line of defense

- Develops in response to pathogens
- Slower to respond
- Specific targets
- Creates memory

2'-FL HMO

Inflammatory cytokines

Innate immune cells

Antibodies

T and B cell subsets

Lymphocyte proliferation

Cytokine production

Nucleotides

Male D, et al. *Immunology.* 2013;3-31. Albers R, et al. *Br J Nutr*. 2013; 110 Suppl 2S1-30.

WHAT ARE NUCLEOTIDES

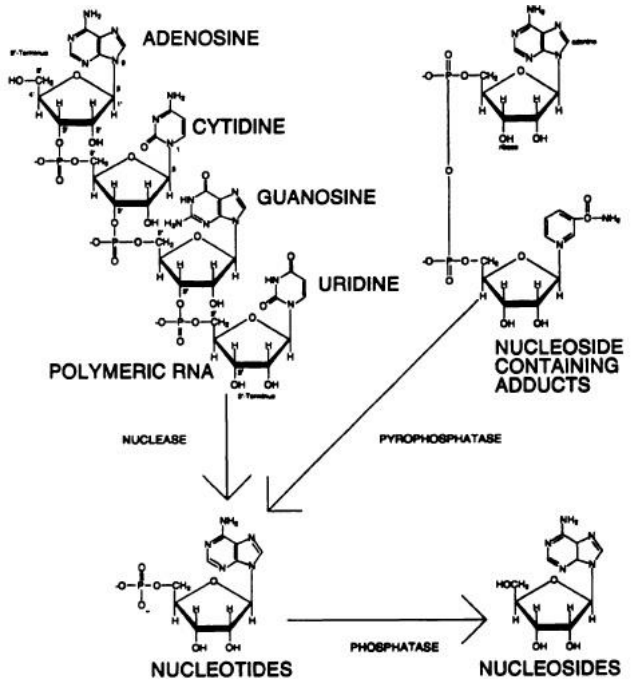
- Important building blocks for RNA and DNA, found in cells
- Involve in essential metabolic reactions in cells, energy transfer, and as messenger molecules
- Rapidly growing infants use nucleotides to meet essential nutritional requirements for protein synthesis and cell growth
- Rapid development of immune and GI systems requires a constant supply of nucleotides

Fun fact:
How many days does it take for gut epithelial cells to be totally replaced?
2-3 days / 2-3 weeks / 2-3 months

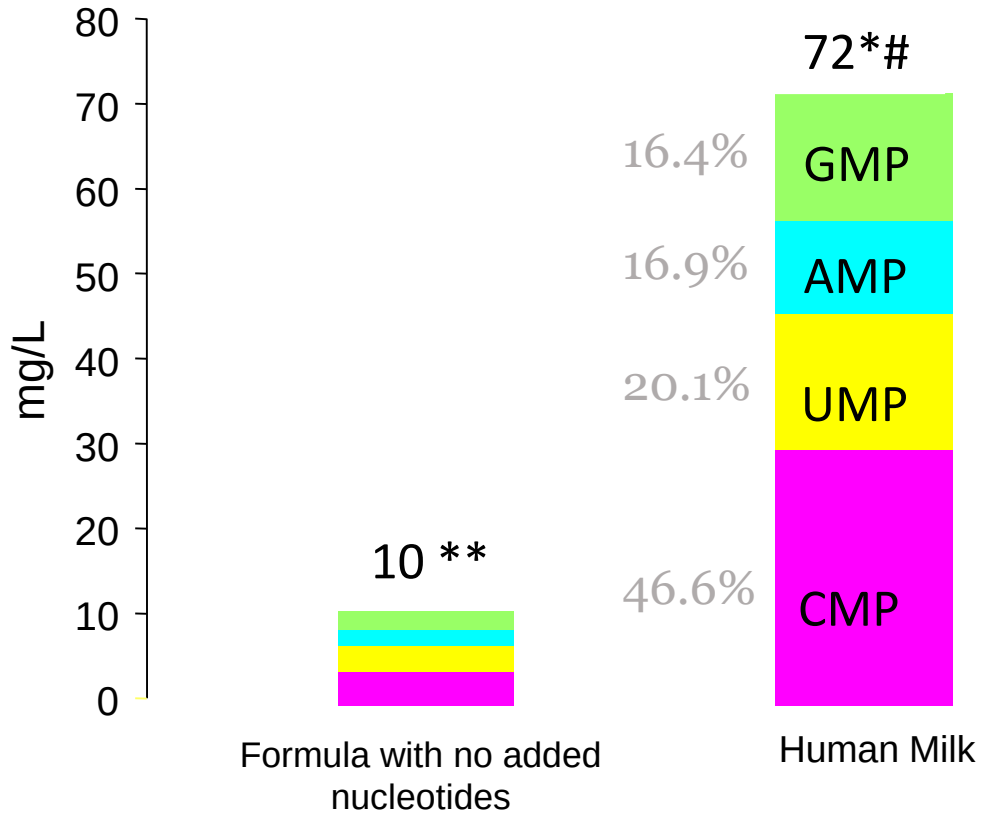
A healthy person will have new gut epithelial/liner cells every 2-3 days

Total Potentially Available Nucleosides/Nucleotides

- Human milk contains free nucleotides, nucleosides, polynucleotides (including RNA) and nucleoside-containing adducts (such as UDP-glucose) that can be digested by nucleases and phosphatases and absorbed as nucleosides.
- TPAN:
- Free nucleotides
- Nucleosides
- Polymeric RNA
- nucleoside-containing adducts



NUCLEOTIDES ARE FOUND TO BE ABUNDANT IN HUMAN MILK

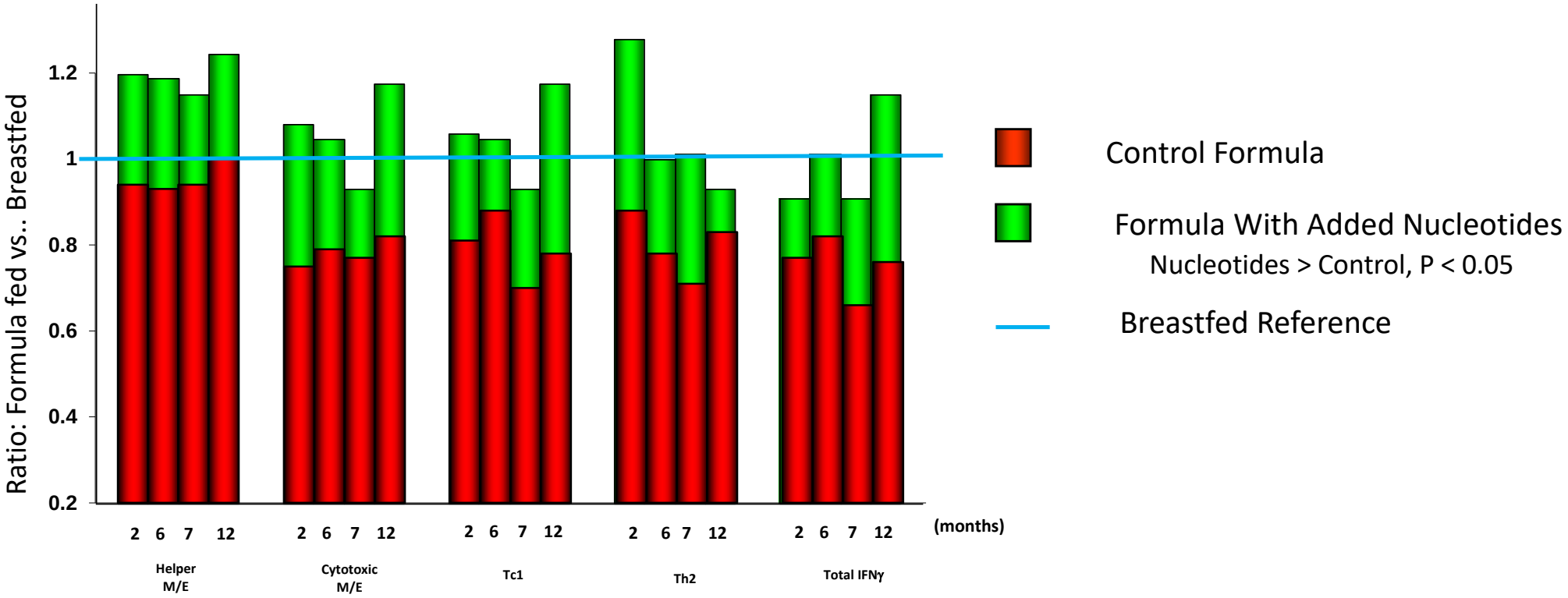


*These nucleotides are potentially available. The full extent of bioavailability has not been determined.

** Inherent level of total nucleotides is approximately 10 mg/L.

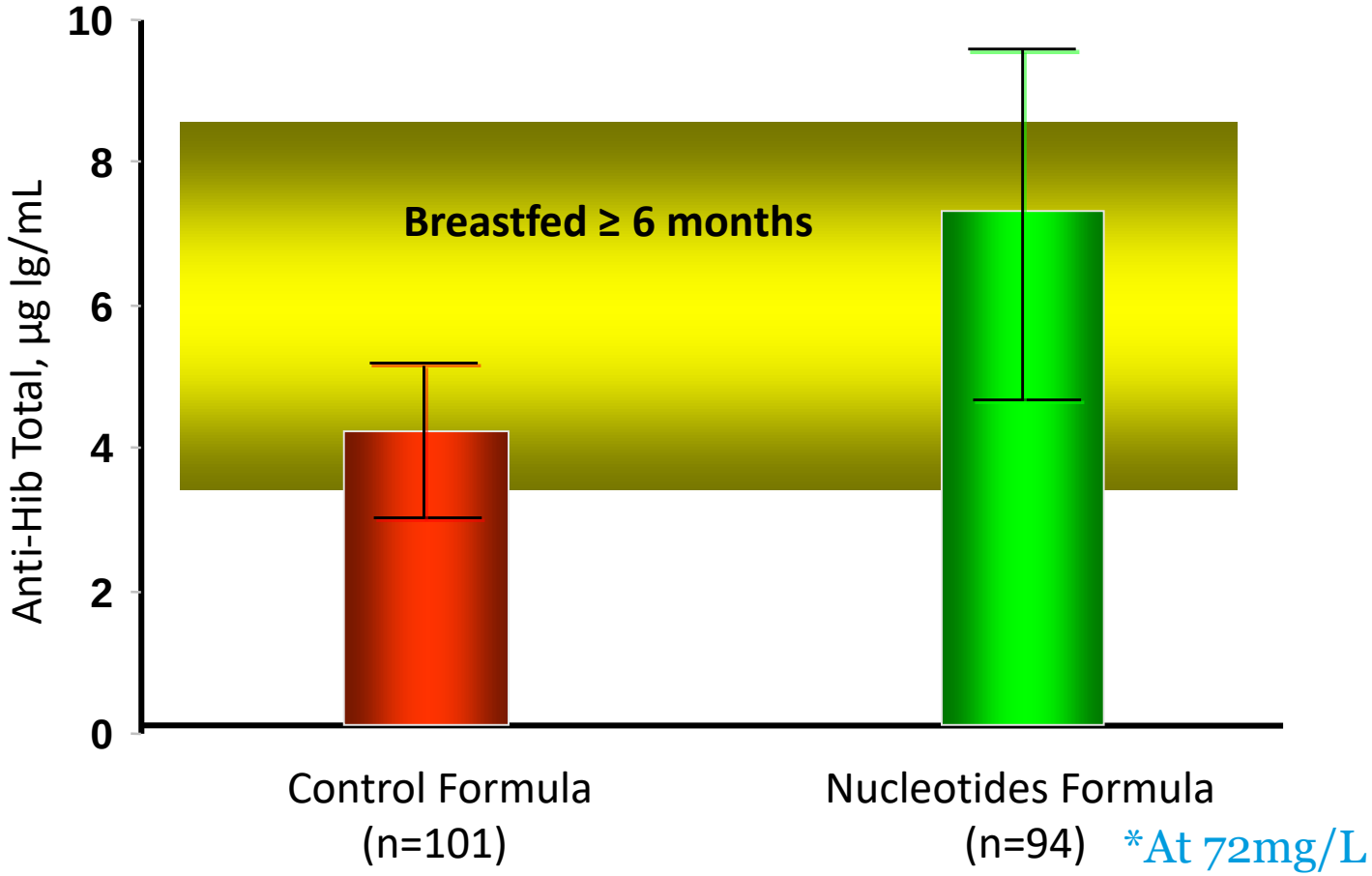
#Leach et al. (1995); Tressler et al. (2003).

NUCLEOTIDES PROMOTE T CELL MATURATION LIKE BREASTFED INFANTS



Buck et al. *Pediatr Res*. (2004); Schaller et al. (2007)

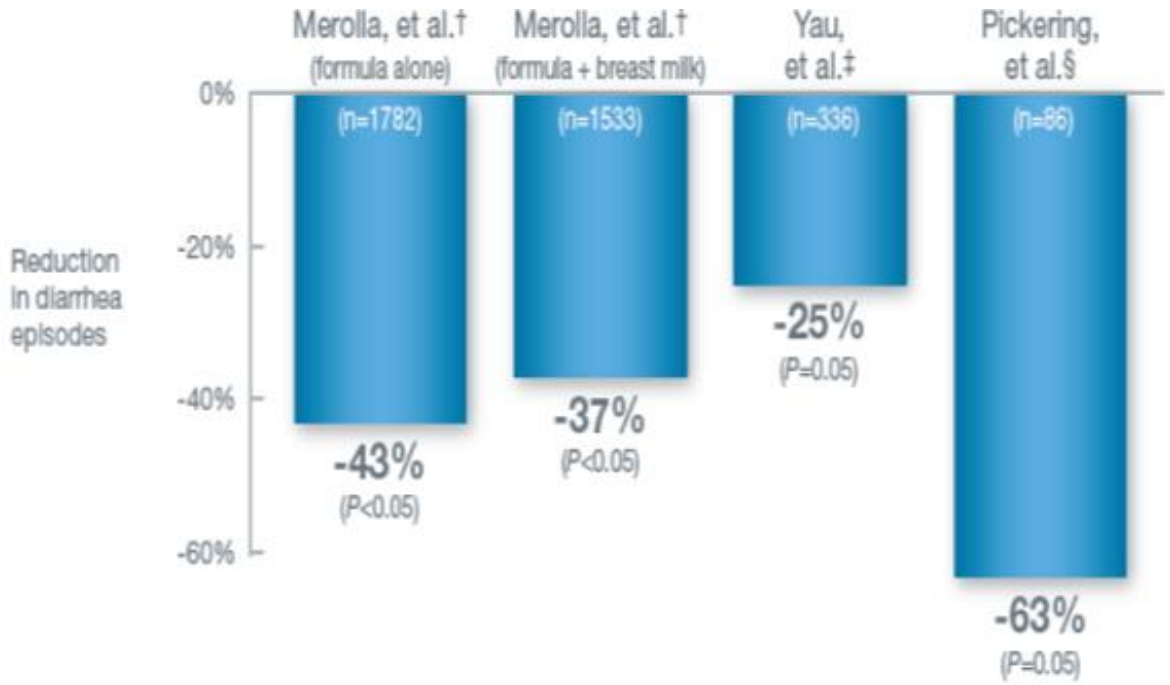
SUPPLEMENTING BABIES WITH TOTAL POTENTIALLY AVAILABLE NUCLEOTIDES INCREASED VACCINE RESPONSE



Nucleotides > Control, P < 0.01

Pickering et al., *Pediatrics*, 1998.

NUCLEOTIDES REDUCE DIARRHEA



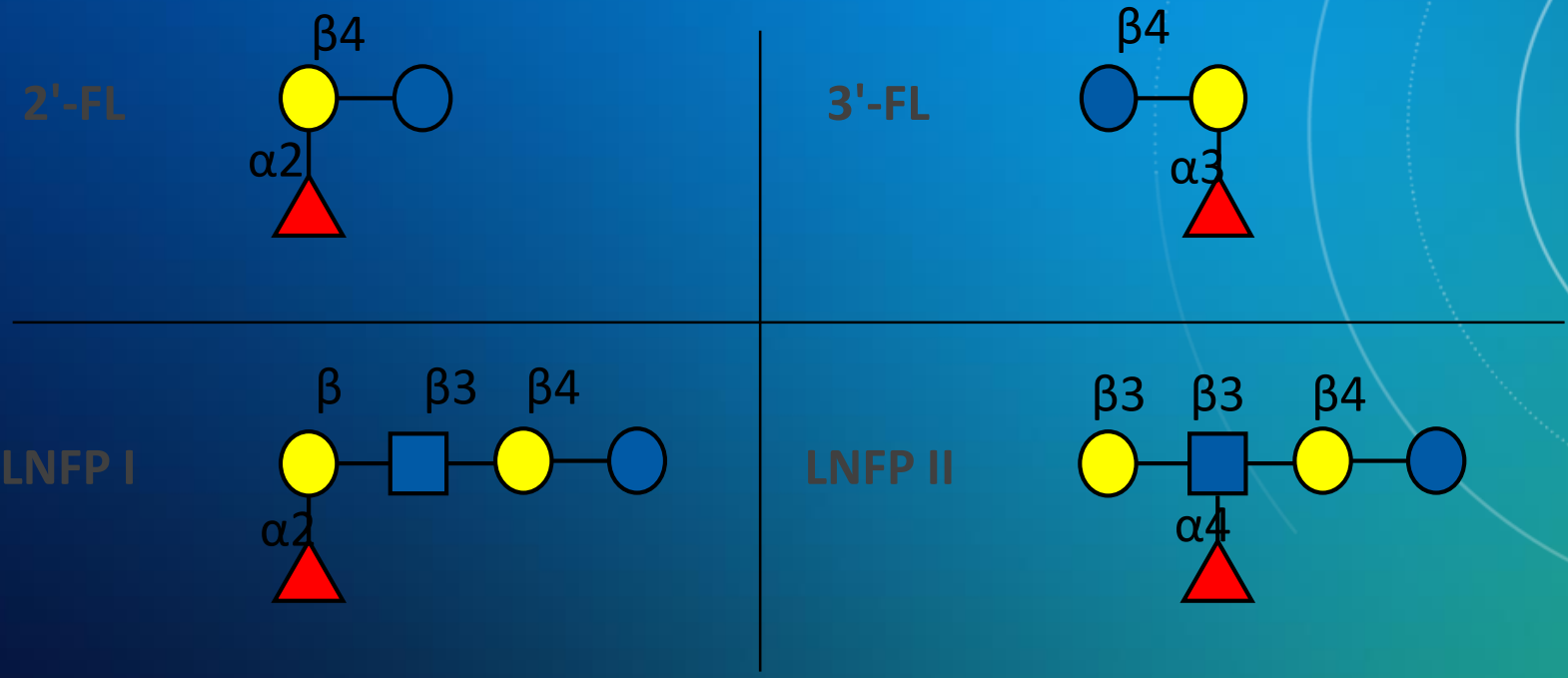
* Reduction based on first diarrheal episode measured in healthy infants. Diarrhea defined as ≥3 watery/loose stools in one 24-hour period
† 0 to 6 months
‡ There was a 14% reduction in the primary endpoint, diarrhea from 8 to 48 weeks (*P*=0.06)
§ Day 4 to Week 52

Nucleotide supplementation lead to **25-63%** reduction in diarrhea episodes in **3 clinical studies involving over 3700 infants**

Merolla et al. Minerva Pediatrica. (2000); Pickering et al. Pediatrics. (1998); Yau et al. J Pediatr Gastroenterol Nutr. (2003)

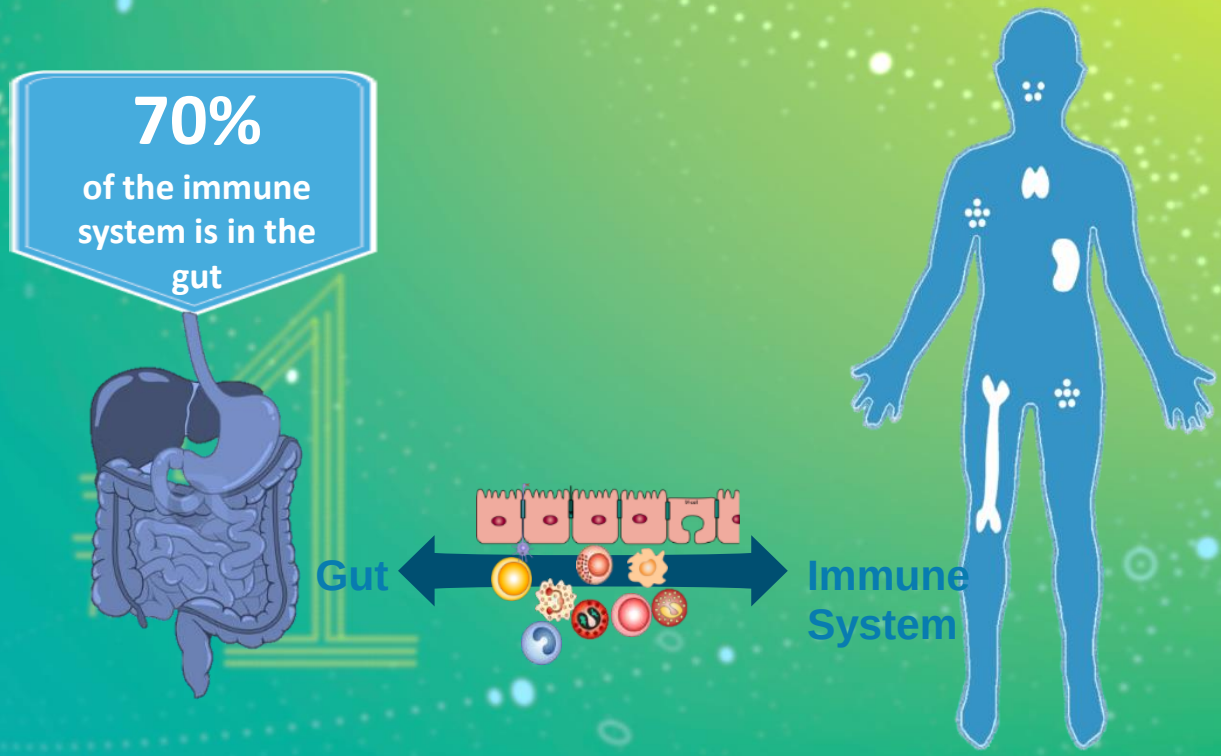
Next frontier in Pediatric Nutrition

Human Milk Oligosaccharides



Gut is an important immune system

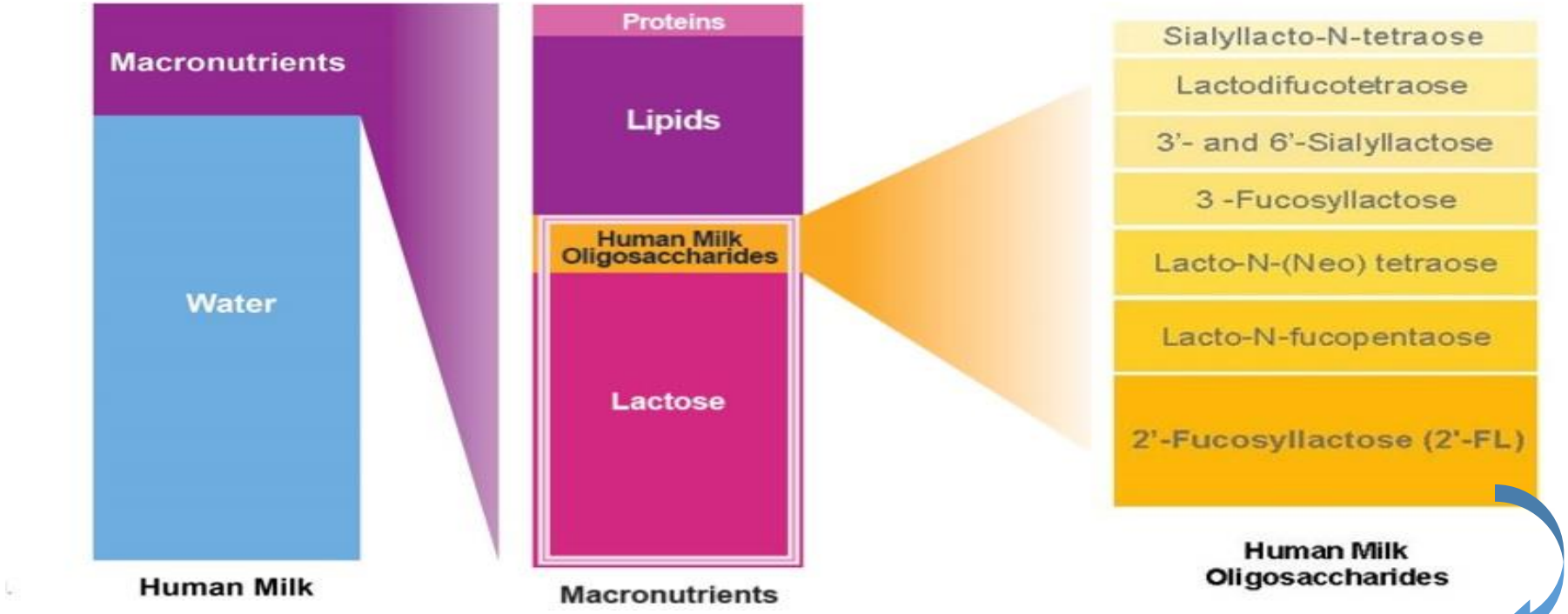
- Babies gut is not matured, and sensitive
- 70% of the immune system is in the gut
- may increase the risk of allergy due to poor immune responses



Furness 1999. Am J Physiol

HMOs Are Abundant in Human Breast Milk

HMOs are the 3rd most abundant component of human breast milk (~ 5-20 g/L) after lactose and lipid, higher than protein



2'FL is the most abundant HMO in most of the mothers' breast milk: 0.06 – 4.65 g/L

McGuire et al. Am J Clin Nutr. (2017); Castanys-Munoz et al. Nutrition Reviews. (2013); Thurl et al. Br J Nutr. (2010)

2-FL is a Unique Multi-functional HMO

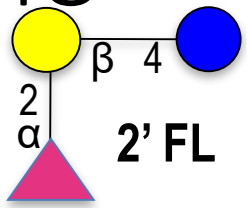
1 HMOs (e.g., 2'-FL) are not digested by human enzymes

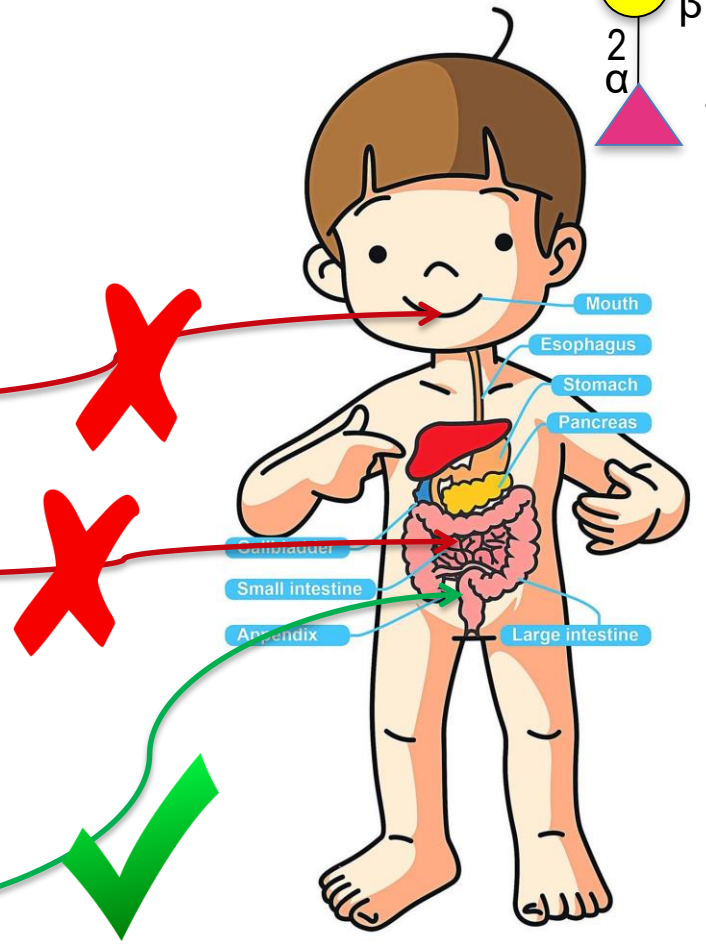
Salivary enzymes do not work

Pancreatic and intestinal enzymes do not work

2'-FL binds to gut pathogens: “receptor decoy”

2 HMOs reach the colon and act as selective prebiotics

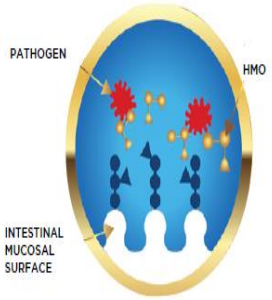




PROMOTING THE GOOD

Helps promote the development of a **healthy microbiota** upon reaching the colon where they act as selective prebiotics

FIGHTING THE BAD

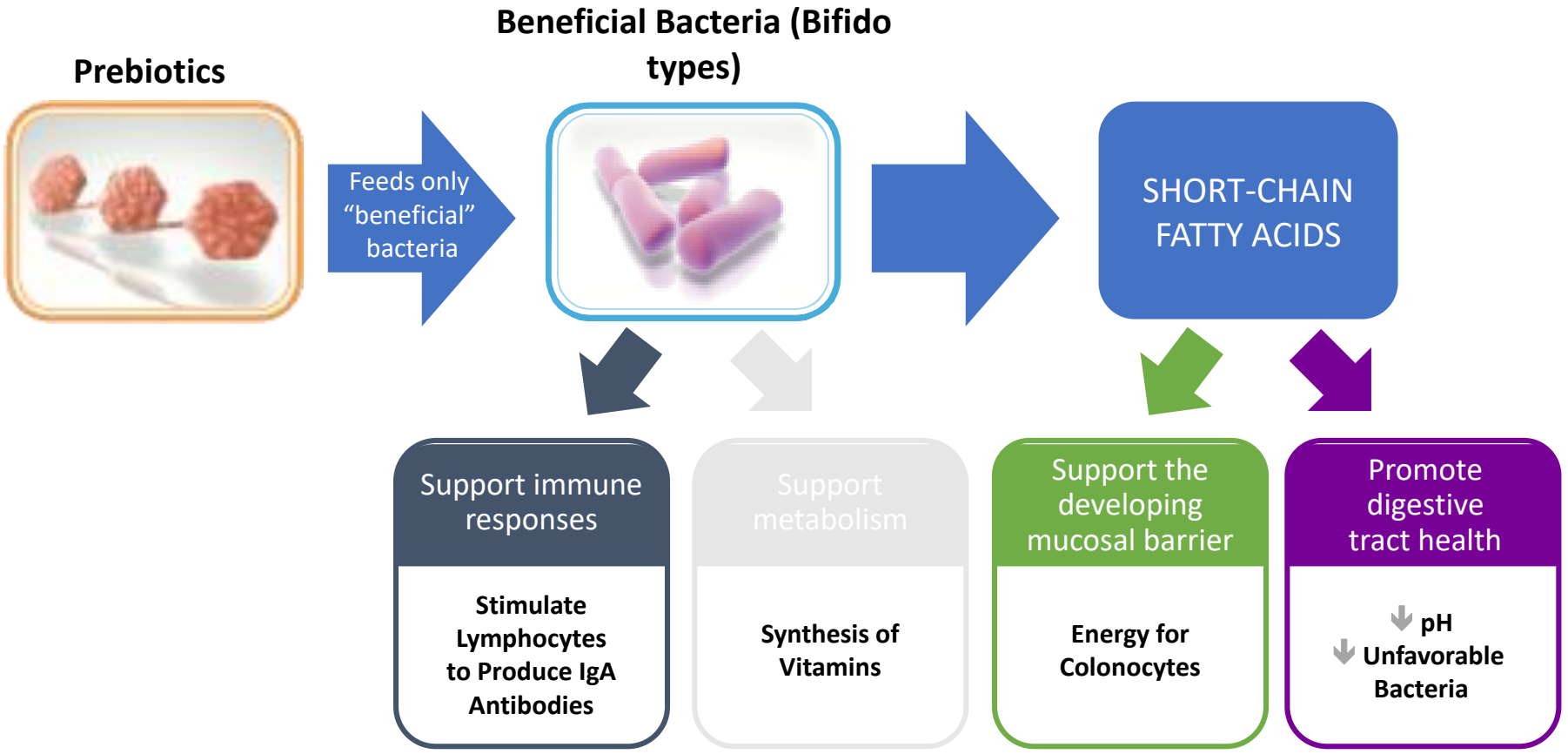


Acts as **receptor decoys** to prevent pathogens adhering to cell surfaces

STRENGTHENING THE IMMUNE

Triggers cells to release **protective factors** and supports the immune system beyond the gut

Oligosaccharides (Prebiotics) Feed Healthy Microbiota



Reference: Underwood et al.

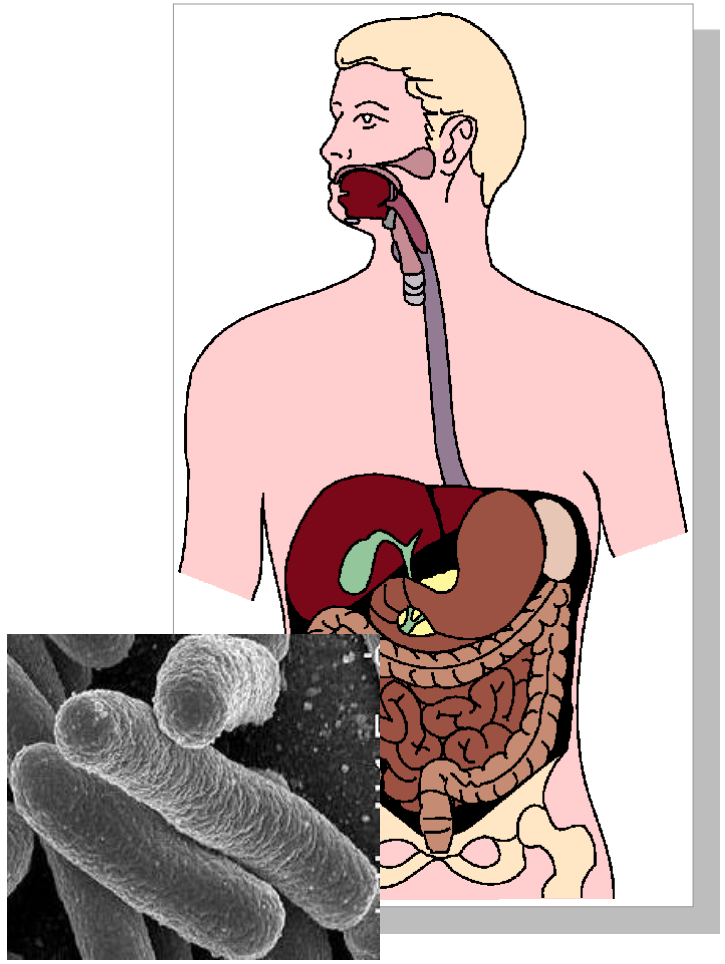
HMO ARE SELECTIVE PREBIOTICS

- Indirectly keeps pathogens in check

✓ Less accommodating for the growth of pathogens

✓ Competitive advantage to non pathogenic commensals(“good bacteria”)

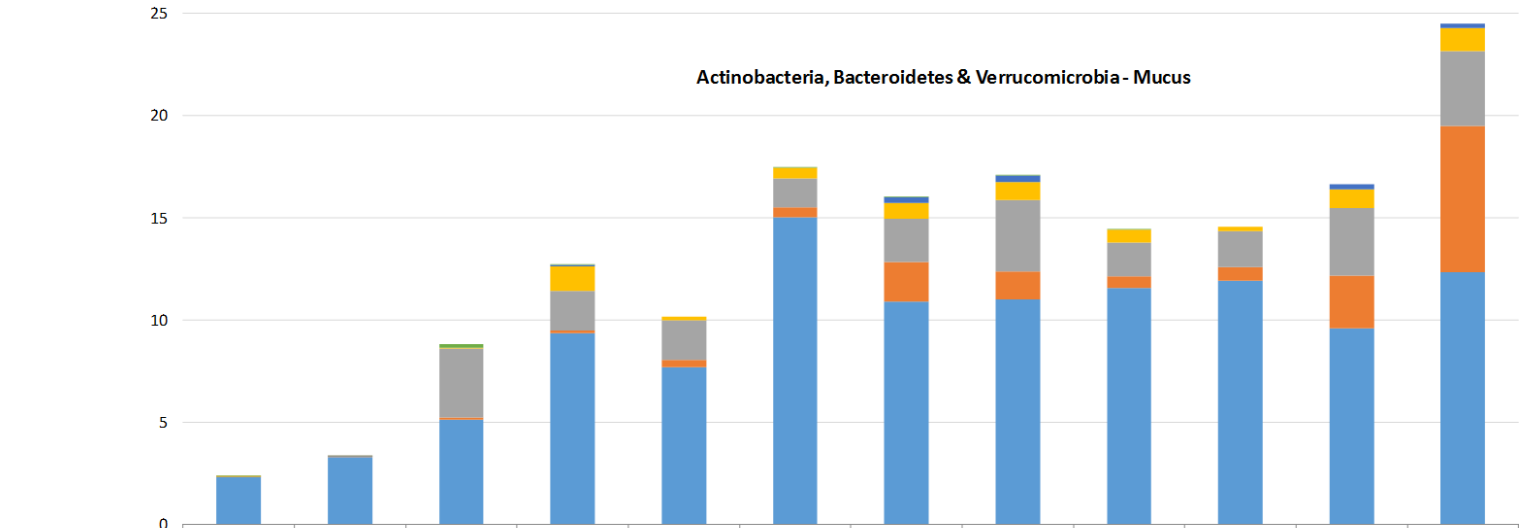
	HMO structure				
Bacterial species (*n*)	2′FL	3-FL	LDFT	3′SL	6′SL
Escherichia coli (1)	−	−	−	−	−
Clostridium (2)	−	−	−	−	−
Lactobacillus (2)	−(1)/+(1)	−(1)/+(1)	−	−	− (1)/++(1)
Enterobacter (2)	−	−	−	−	−
Enterococcus (2)	− (1)/+(1)	− (1)/+(1)	−	−	−
Staphylococcus (2)	−	−	−	−	−
Streptococcus (1)	+	+	−	−	−
Bacteroides (3)	++	++	−(1)/++(2)	−(1)/+(1)/++(1)	+(1)/++(2)
Bifidobacterium (10)	+(1)/++(9)	++	+(1)/++(9)	−(2)/+(1)/++(7)	−(1)/+(1)/++(8)



Bifidobacteria and Bacteroides are better at metabolizing HMOs

Reference: Yu et al. (2013)

2’-FL HMO Alters Composition and Activity of Gut Microbiota

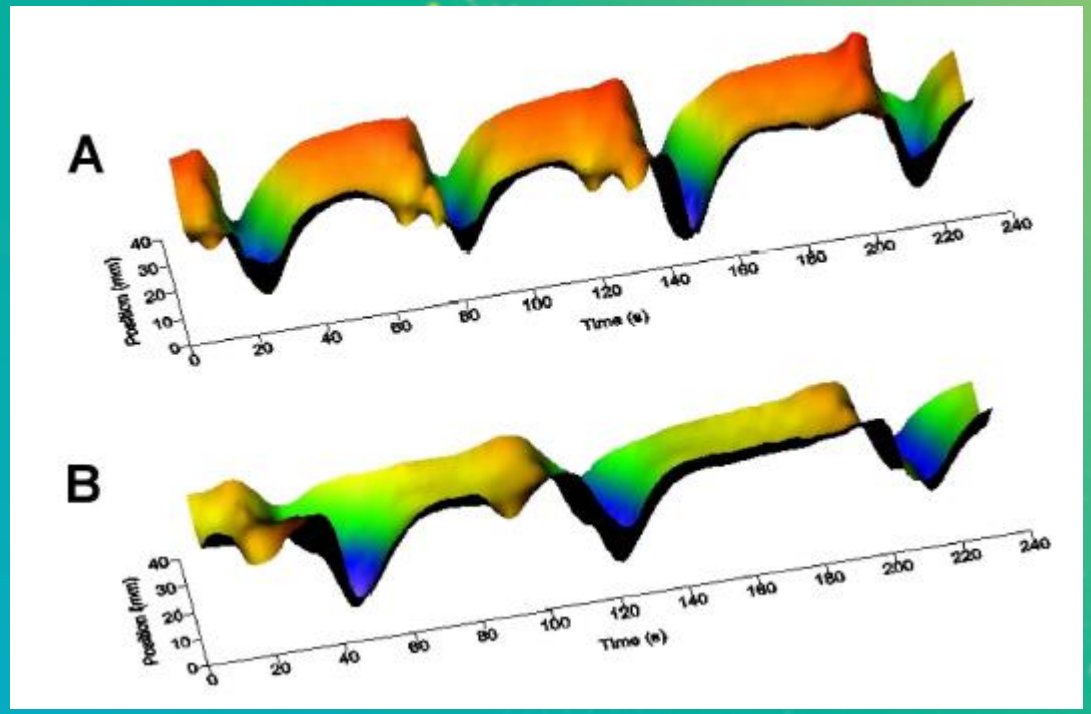


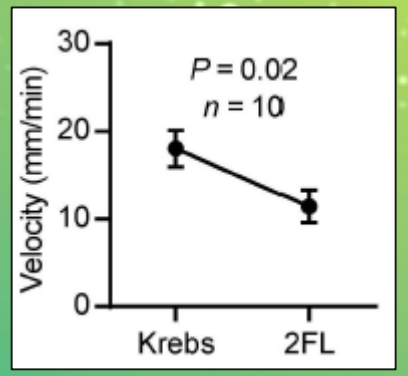
	Donor 1				Donor 2				Donor 3			
	PC		DC		PC		DC		PC		DC	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Verrucomicrobiaceae	0,00	0,00	0,19	0,03	0,00	0,01	0,03	0,02	0,00	0,00	0,00	0,00
Rikenellaceae	0,00	0,00	0,00	0,07	0,00	0,00	0,26	0,33	0,00	0,00	0,23	0,20
Porphyromonadaceae	0,00	0,01	0,01	1,21	0,14	0,53	0,77	0,87	0,65	0,19	0,93	1,15
Bacteroidaceae	0,02	0,02	3,40	1,93	1,94	1,43	2,12	3,51	1,66	1,79	3,30	3,65
Coriobacteriaceae	0,01	0,03	0,10	0,14	0,36	0,47	1,95	1,35	0,57	0,66	2,60	7,15
Bifidobacteriaceae	2,32	3,28	5,13	9,36	7,70	15,03	10,89	11,01	11,56	11,92	9,58	12,34

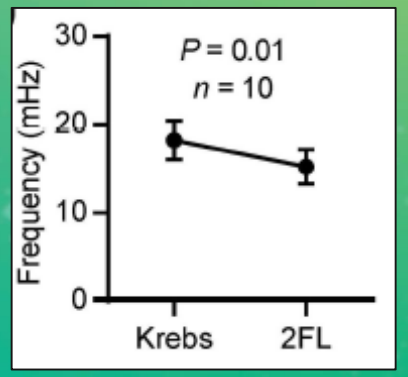
Assessed via 16S-targeted Illumina sequencing during the control and 2’-FL treatment period, both in the proximal (PC) and distal colon (DC) of the TRIPLE-baby SHIME® that simulated the gut microbiota of babies.

Reference: Abbeele et al. (2019)

2’-FL HMO Improves Gut Health by Diminishing Colon Motor Contractions







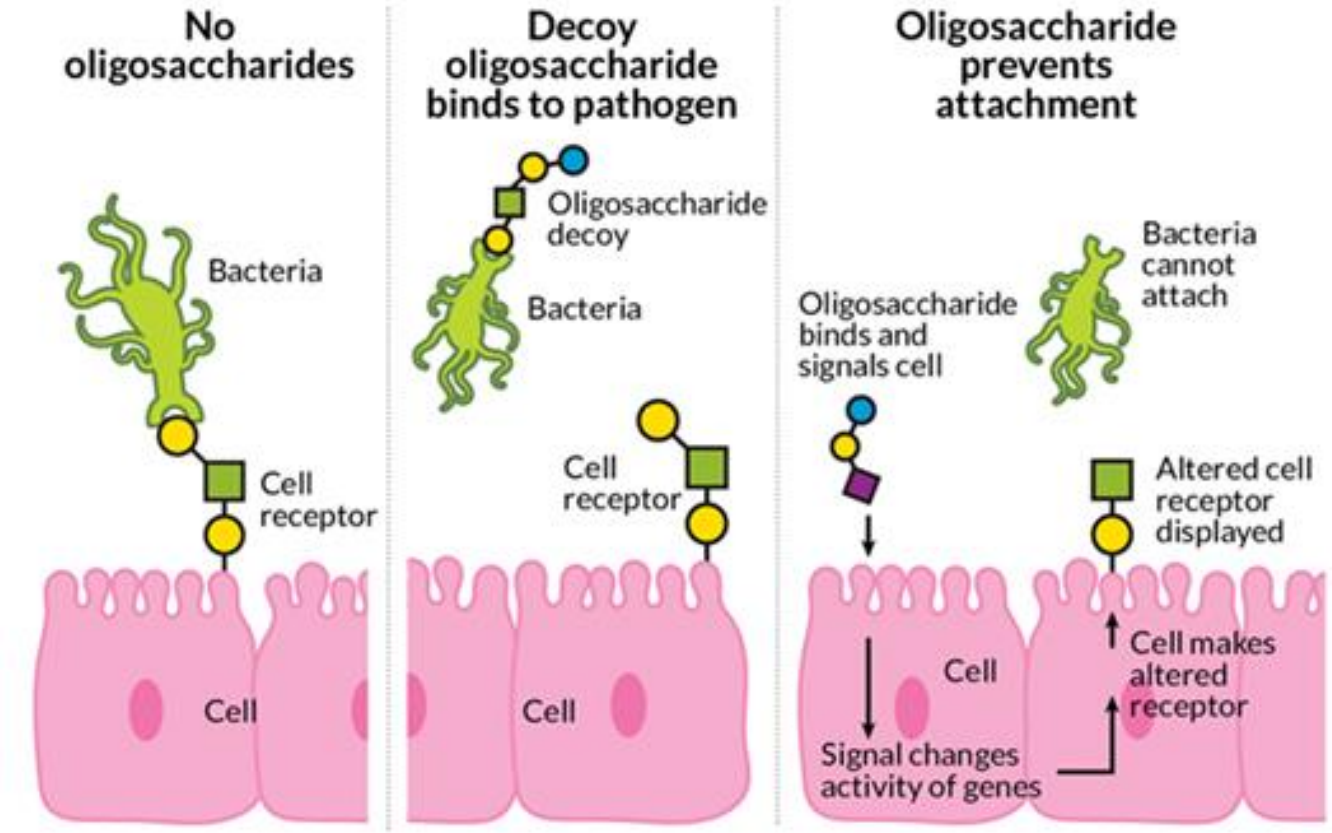
- 2’-FL HMO reduced velocity & frequency of colon contractions (migrating motor complexes, MMCs), a condition which may cause stomach discomfort

*Pre-clinical study in mice

Reference: Bienenstock et al. (2013)

HMOs PREVENT PATHOGEN ADHERENCE AS RECEPTOR DECOYS

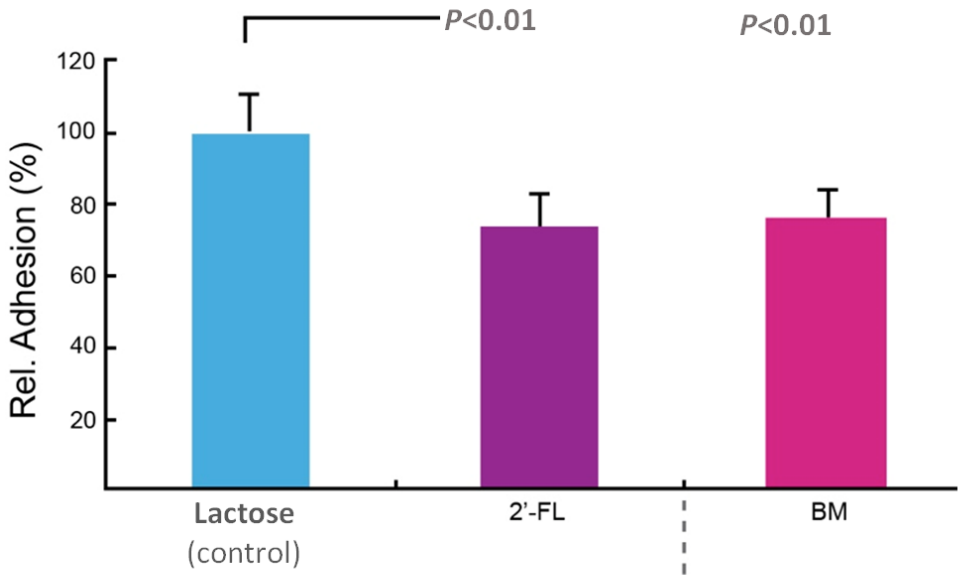
HMOs may prevent pathogen adherence to cell surfaces by acting as receptor decoys



Bode L. *Glycobiology*. 2012;22:1147–1162.

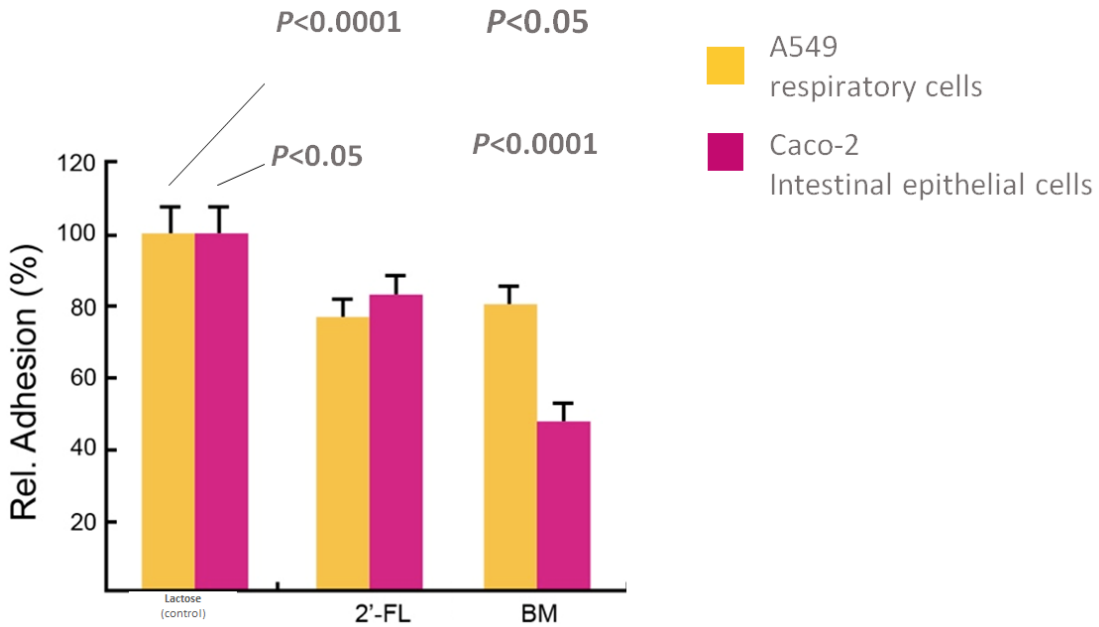
HMOs Inhibited Adhesion of Pathogens

Significantly reduced adhesion to intestinal epithelial (Caco-2) cells



C. jejuni (10 mg/mL)

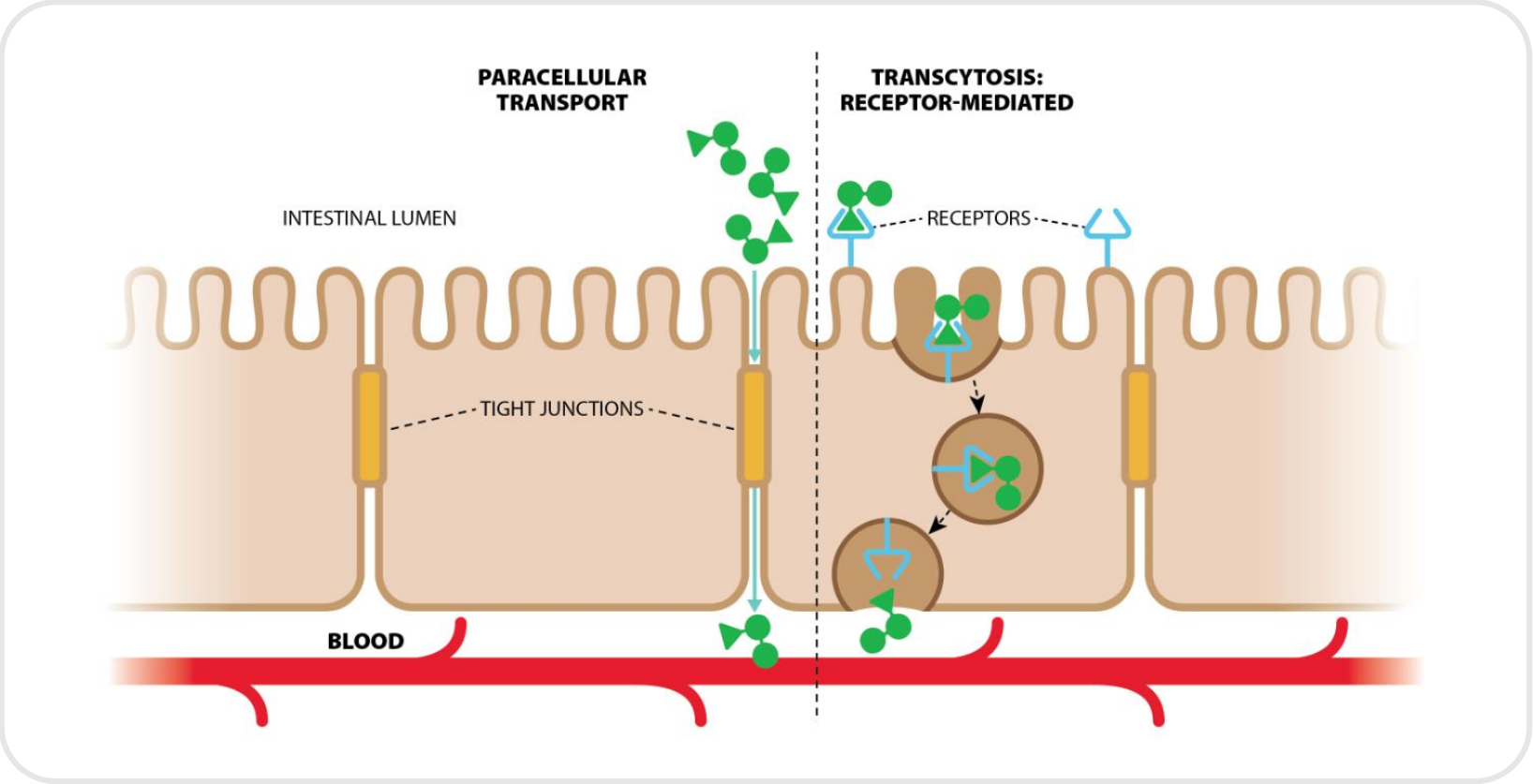
Significantly reduced adhesion to respiratory (A549) and epithelial cells (Caco-2)



P. aeruginosa (10 mg/mL)

(*in vitro* study)

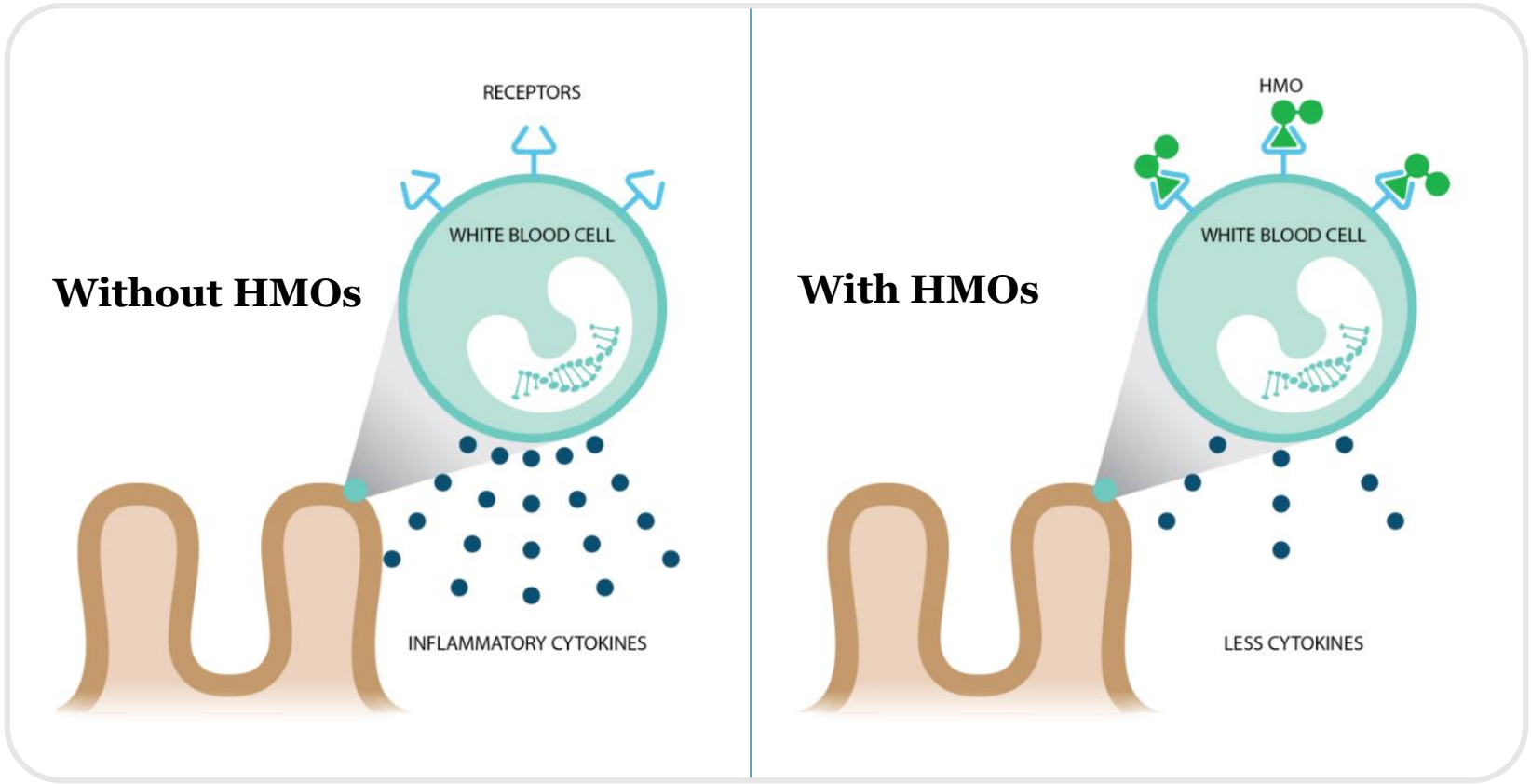
HMOs Cross Gut Epithelia[1,2]
STRENGTHEN IMMUNITY BEYOND THE GI TRACT



1. Gnoth MJ, et al. *J Biol Chem.* 2001;276:34363–34370. **2**. Gnoth MJ, et al. *Food Res In*t. 2002;35:145–149.

HMOs STRENGTHEN IMMUNITY BY REGULATING THE RELEASE OF PROTECTIVE FACTORS

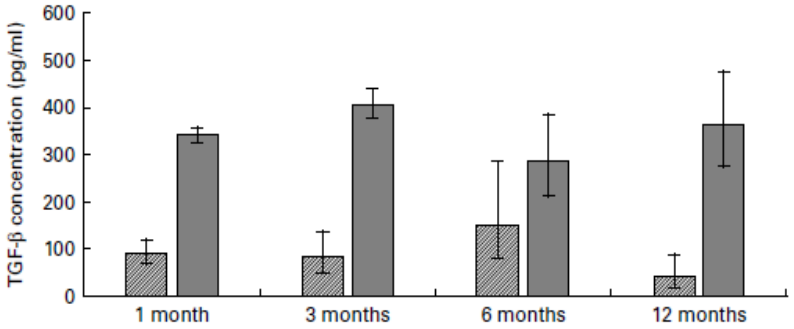
- HMOs may bind to cell surfaces and regulate the release of protective factors



Breastfed babies had better inflammatory cytokines profile vs formula-fed babies in high risk population

anti-inflammatory cytokines

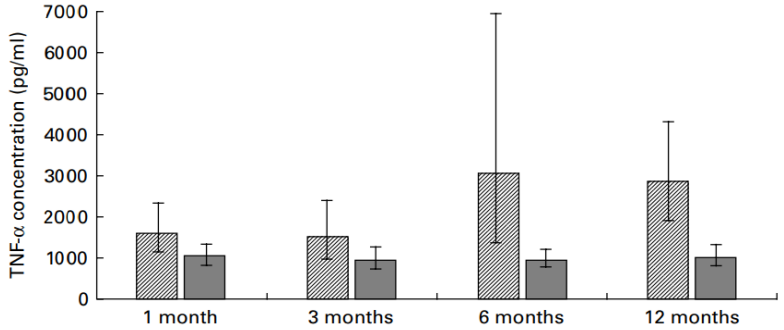
TGF-β



Breastfed babies had higher anti-inflammatory responses

Pro-inflammatory cytokines

TNF-α

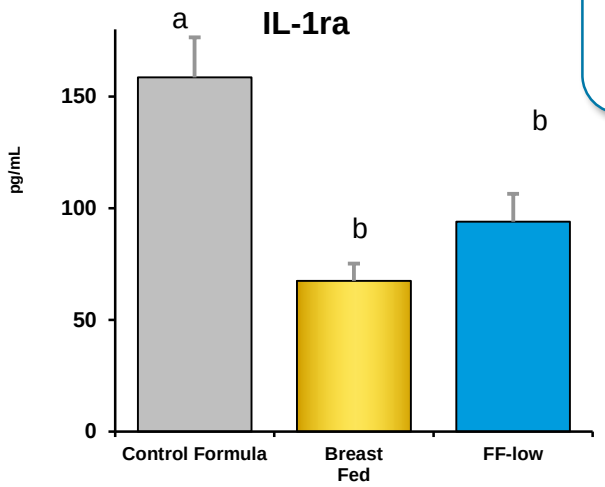


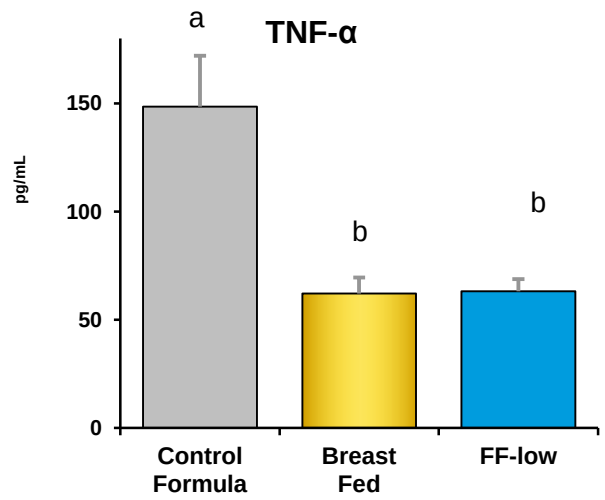
Breastfed babies had lower pro-inflammatory responses

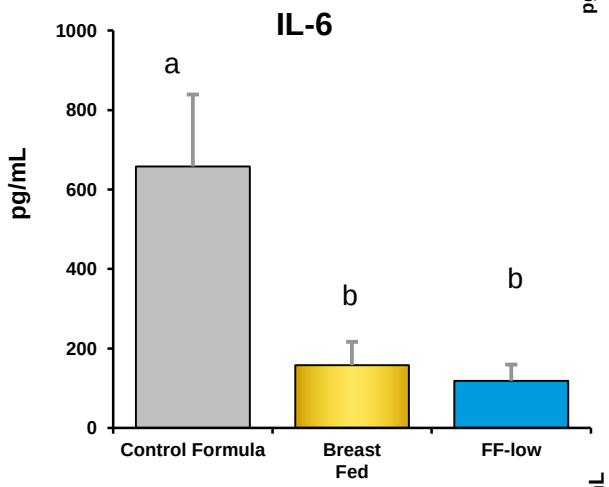
Reference: Kainonen et al. (2013)

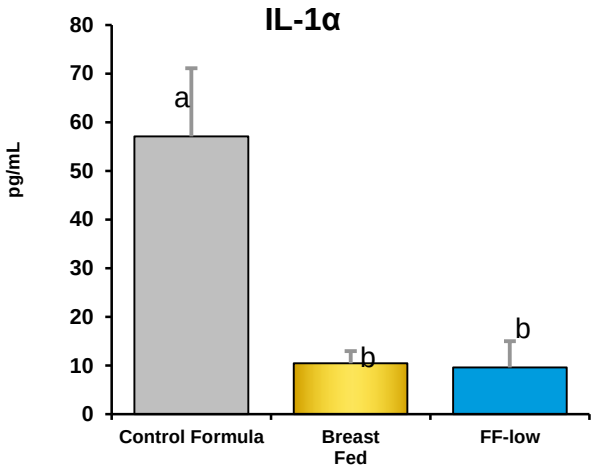
2’-FL Regulates Inflammatory Cytokines in Plasma

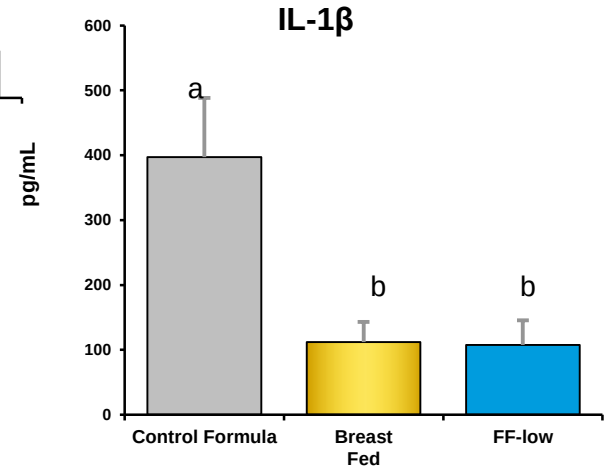
2′-FL HMO Closed Gaps between Breast Fed and Formula Fed











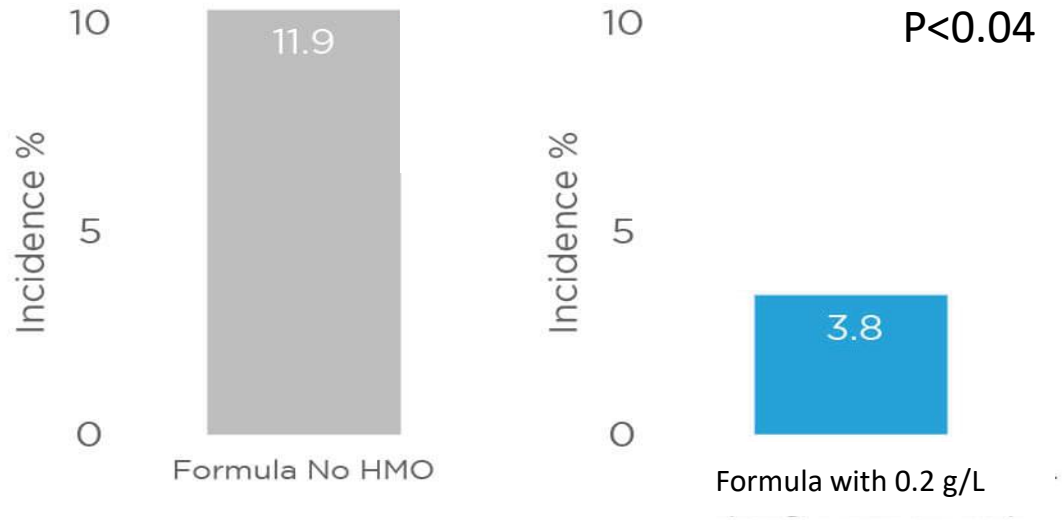
Mean±SEM;
Means without a common letter differ, **P≤0.05**

	Control Formula (no 2’-FL)	Breast fed	FF-low (0.2 g/L 2’-FL)
N*	39	42	37

*Intent to treat

Goehring KC, et al. *J. Nutr.* 2016;146(12):2559-2566.

HMO Supplementation to Babies for 4 months was associated with Fewer infections

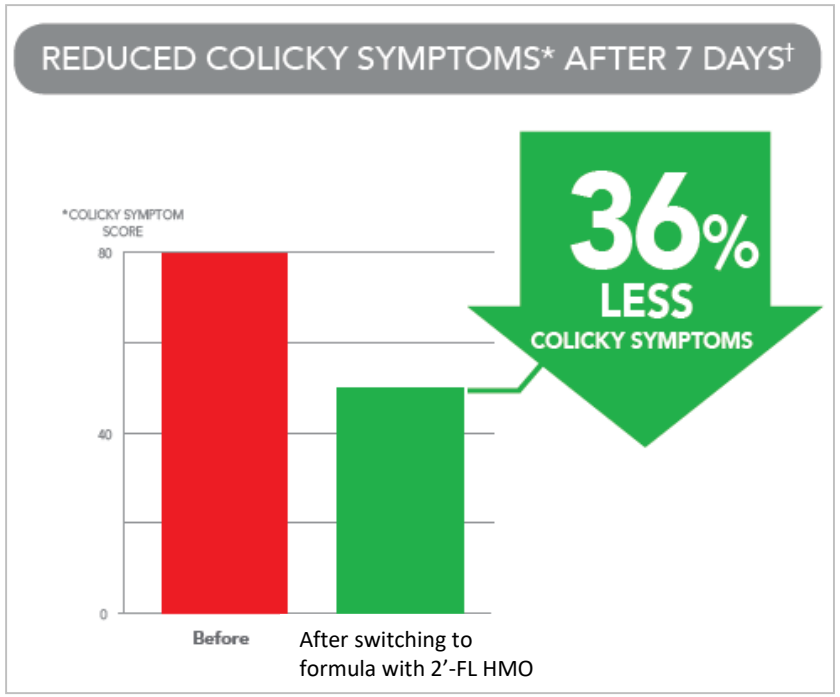


P<0.04

Parent reported and confirmed by study physician, Reverri et al. (2018)

HMO is associated with reduced colicky symptoms

adverse event reporting-based claim



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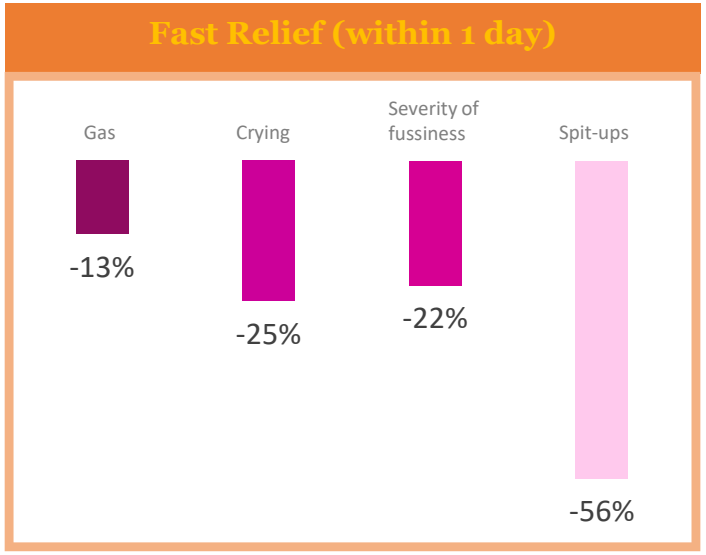
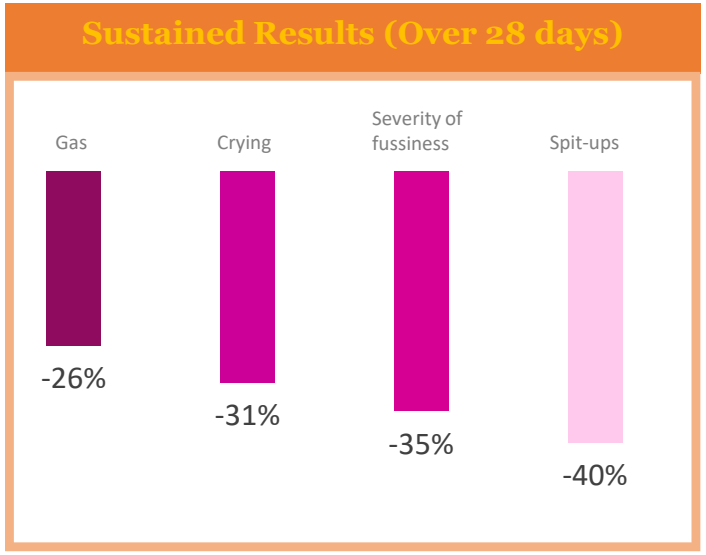
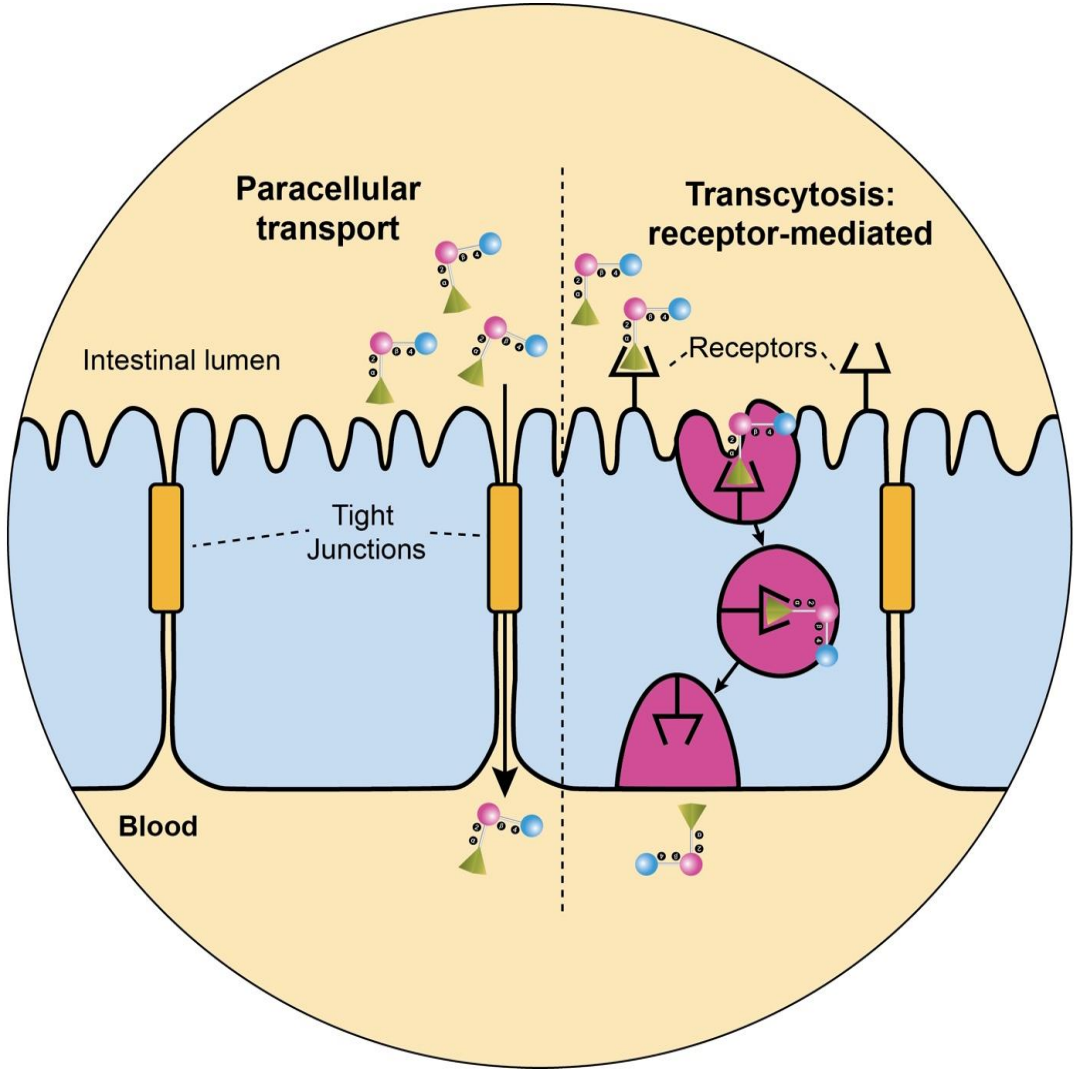




Reverri et al. (2018)

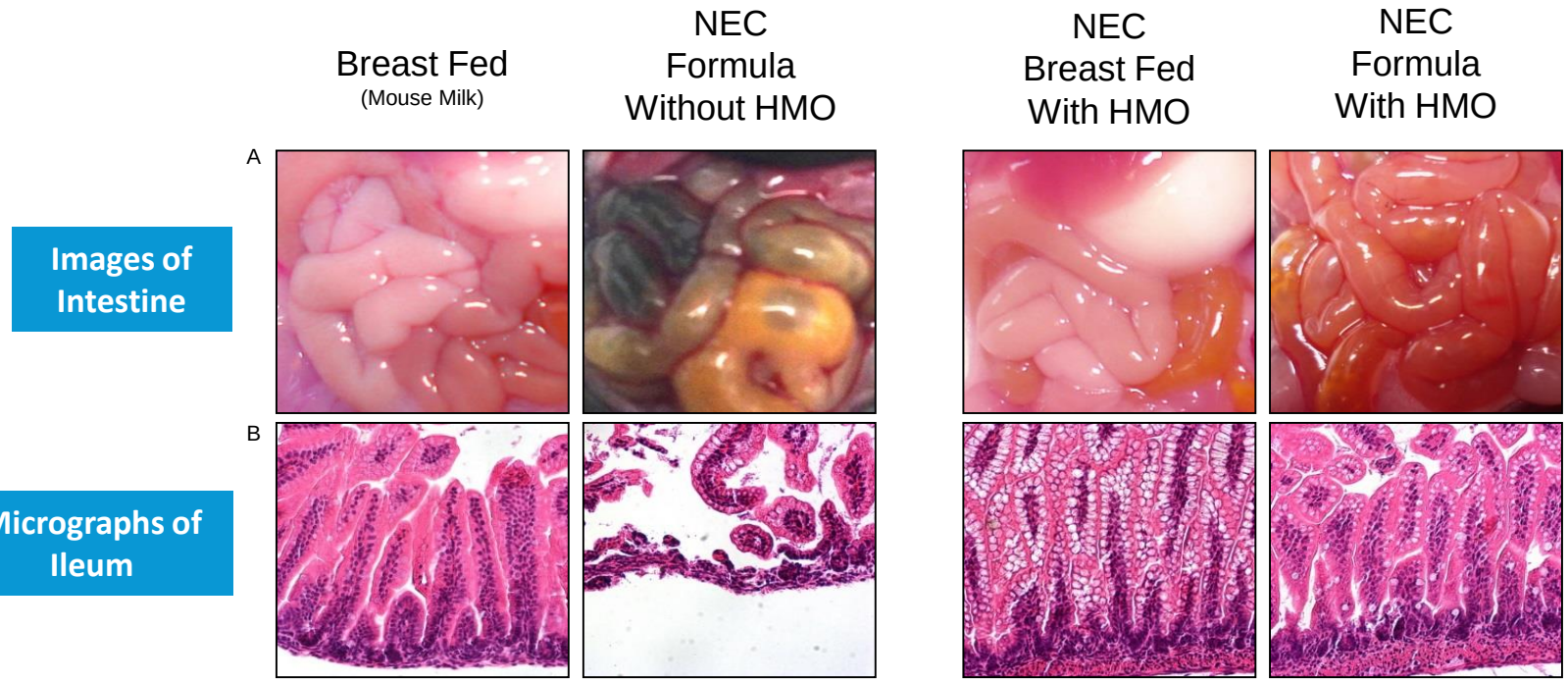
Metabolism of HMO—Beyond Gut



HMOs have been shown to be able to cross gut epithelia and enter circulation, thus exert their effect beyond gut

Gnoth 2001 and 2002

Preclinical Study in Mice indicated that 2’-FL HMO attenuates the severity of Necrotizing enterocolitis (NEC)

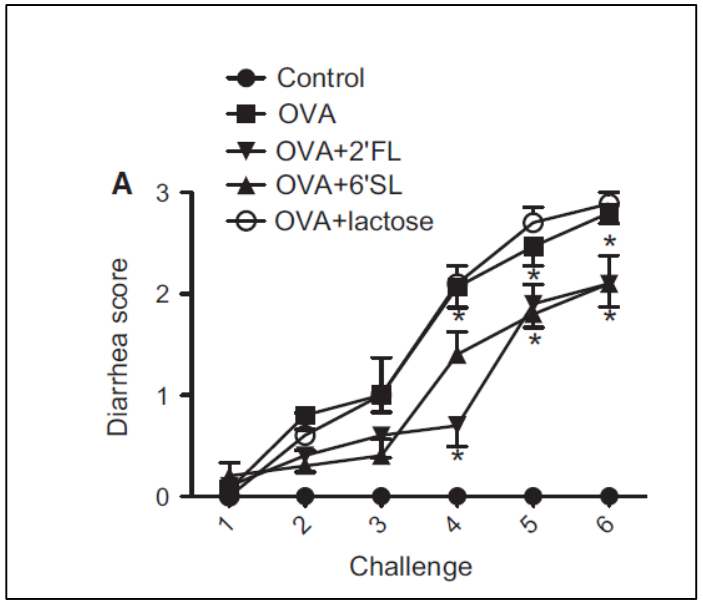


- 2’-FL decreases pro-inflammatory markers of NEC
- 2’-FL helps preserve the mucosal architecture of the small intestine
- 2’-FL upregulates the vasodilatory molecule which helped maintain intestinal perfusion

Good et al. Br J Nutr. (2016)

2’-FL attenuates food allergy symptoms in rat model & human intestinal epithelial cell lines

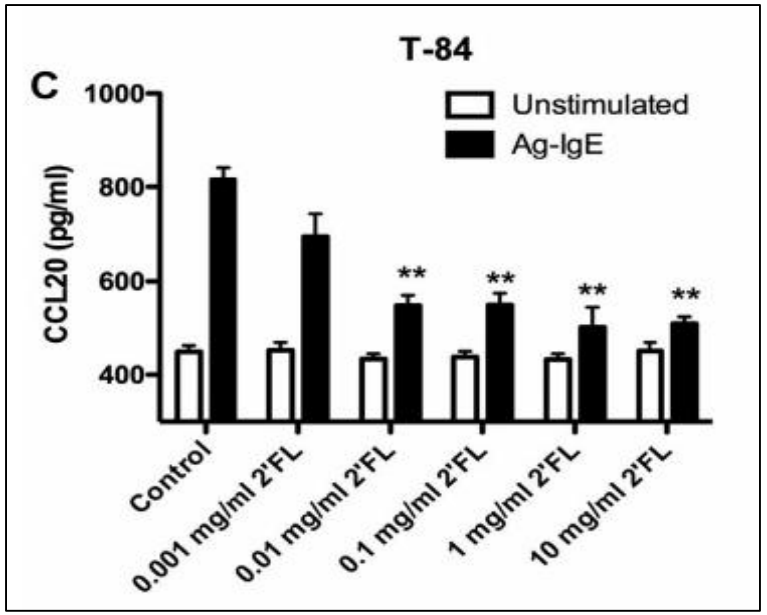
Rat model



The diarrhea scores were significantly lower in HMO groups compared to control group

Castillo-Courtade et al. Allergy (2015)

Intestinal epithelial cell model



The release of CCL-20 (pro-inflammatory chemokines) were significantly lower in 2’-FL HMO groups compared to control group

Zehra et al. JFDS. (2018)

2’-FL HMO affects cognitive domains and improves learning and memory in rodents

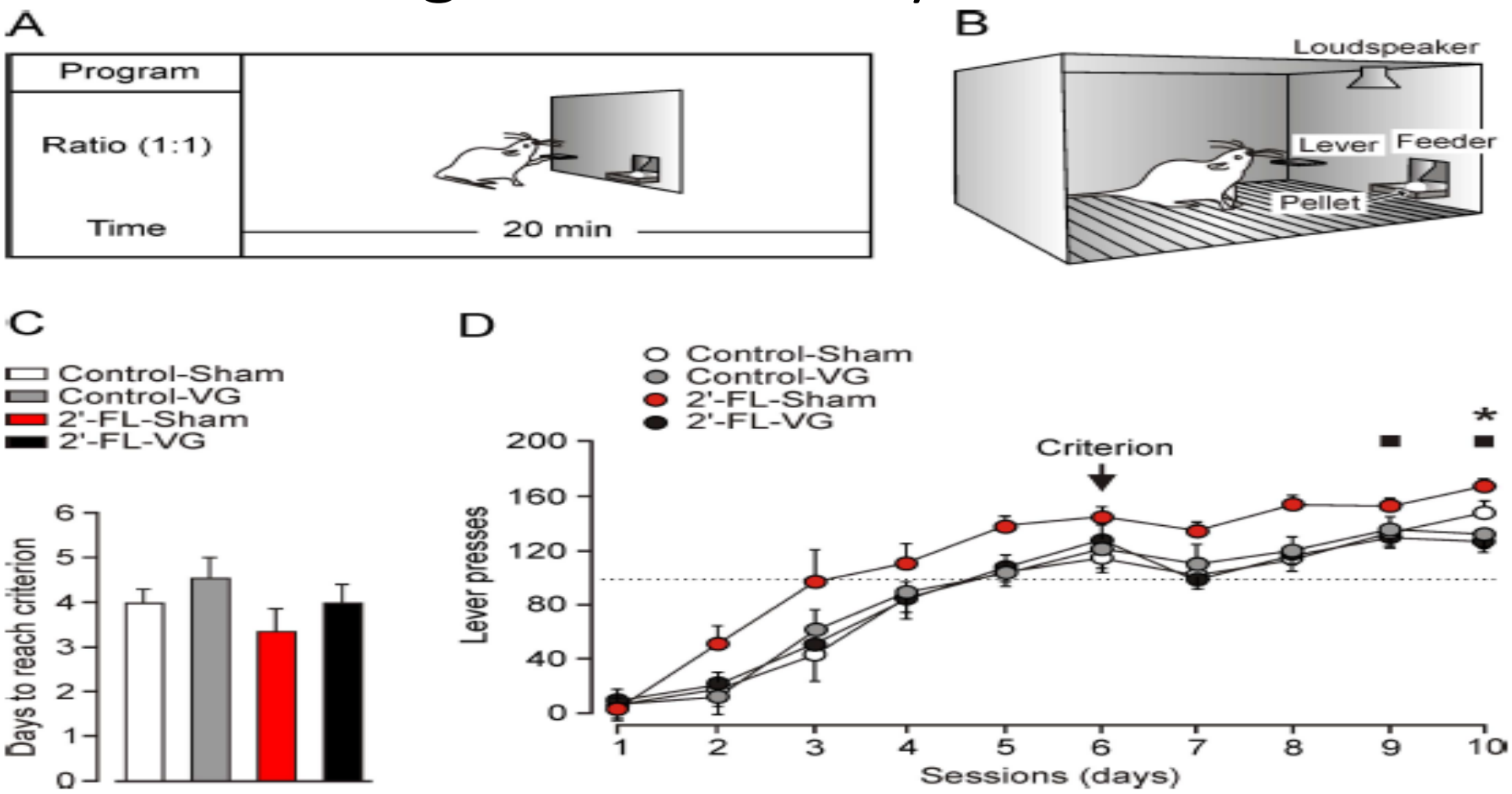


Fig 2. Chronic administration of 2’-FL potentiates the acquisition of an operant conditioning task in behaving rats, but this positive effect was prevented by a bilateral vagotomy. (A,B) Four (Control-

Vazquez et al. PLOS ONE. (2016)

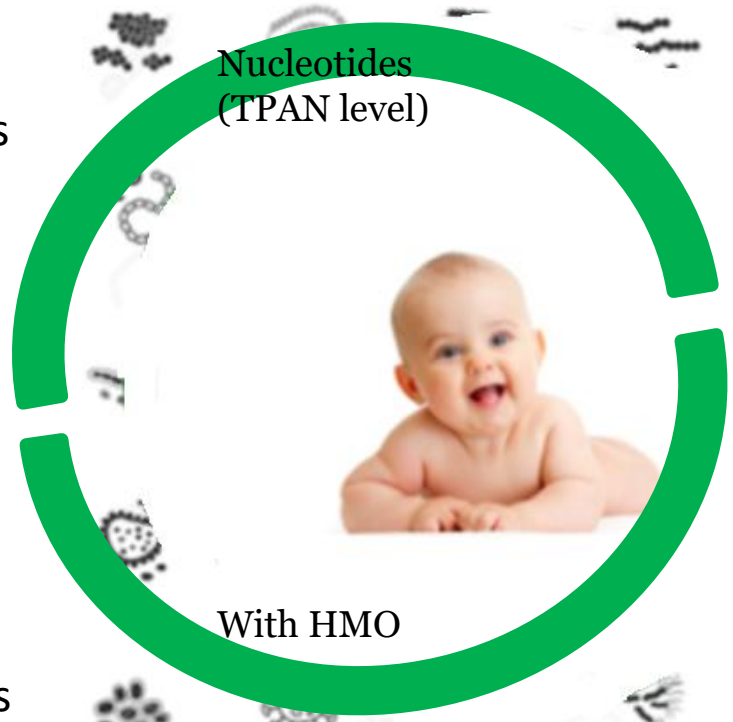
Dual Protection for Babies for Strong Immunity

Nucleotides at TPAN level

- Strengthen gut epithelium barriers
- Promote antibody production from B cells
- Promote T cell maturation

HMO

- Strengthen gut epithelium barriers
- Supports healthy gut microbiota
- Regulates inflammatory cytokines



May Every child flourish