USF HEALTH COLLEGE OF NURSING
UNIVERSITY OF SOUTH FLORIDA

The Adaptability of Milk Molecules: Maternal-Infant Interactions

Culture of Breastfeeding Conference
February, 2016

University of South Florida College of Nursing, Tampa, FL

The question

- Does human milk volume and composition through programmed and patterned neuroendocrine pathways/reflexes and independently of maternal-infant sensorineural and behavioral interactions?

What we know

- Stress can inhibit the oxytocin surge and milk let down can be impaired
- Lactating mothers can produce volumes of milk when they think of their ba their baby cry, etc.

Infants can differentiate milk by smell

- Infants “choose” their own mothers’ milk, turning toward the milk produced by their mother and averting from other mothers’ milk

In the Wild

- Animal mothers and infants find each other even in the presence of thousands of distracting smells

So production and volume are affected by sensorineural dyadic patterning

But what about milk composition? The molecules in milk? Is milk a “designer product”?

Study 1:Postpartum Term Mothers and infants

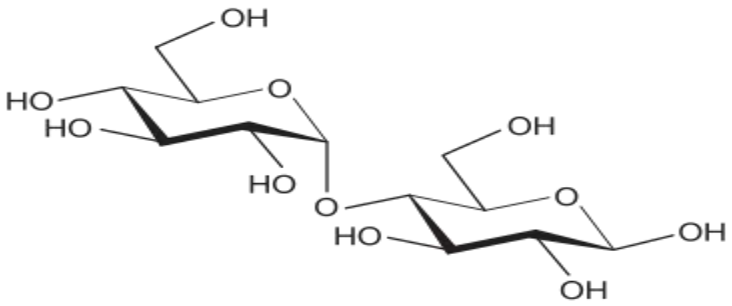
Associations between **human milk** SIgA and maternal immune, infectious, endocrine, and stress variables.
Groer M, Davis M, Steele K. J Hum Lact. 2004 May;20(2):153-8;

Neuroendocrine and immune relationships in postpartum fatigue.
Groër M, Davis M, Casey K, Short B, Smith K, Groër S.
MCN Am J Matern Child Nurs. 2005 Mar-Apr;30(2):133-8.

Stress and Fatigue in the Postpartum

- Stress
  - Roles
  - Physical changes
  - The new baby
  - Relationships
- Fatigue
  - Lack of adequate and continuous sleep
  - Physical exhaustion
  - Daytime sleepiness
  - Metabolic demands

Does this effect milk molecules?



Milk hormones

- **Many hormones** in human milk; function of most is unknown
- Some may be passenger molecules from blood; some may be actively transported.
- Milk Prolactin is associated with immune and neurological development in rodents
- Milk Melatonin in some animal species may play role in regulating infant sleep cycles

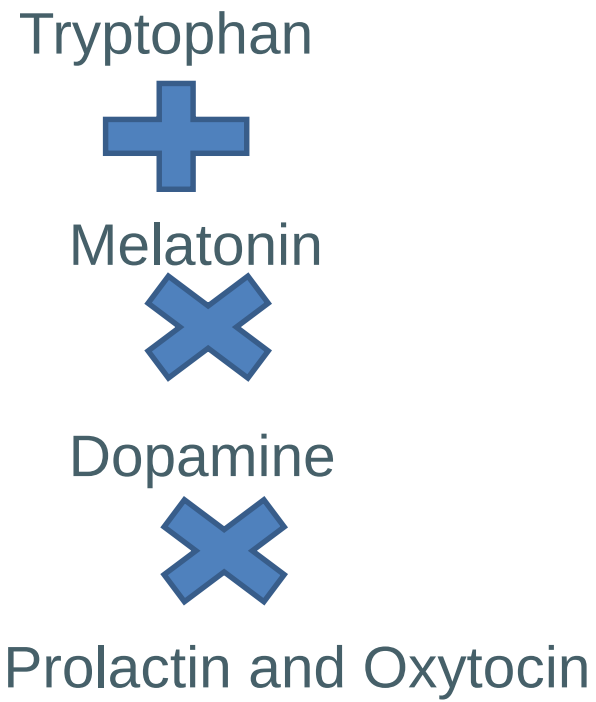

midday / noon
12 midday 12.00
pm
morning
afternoon
DAY
sunrise
sunset
dawn
6 am
06.00
6 pm
18.00
dusk
NIGHT
morning
evening
am
12 midnight 00.00
midnight
© englishclub.com

Postpartum Fatigue

- Is a stressor
- Increases risk for mastitis
- Decreases oxytocin and prolactin
- Associated with postpartum depression

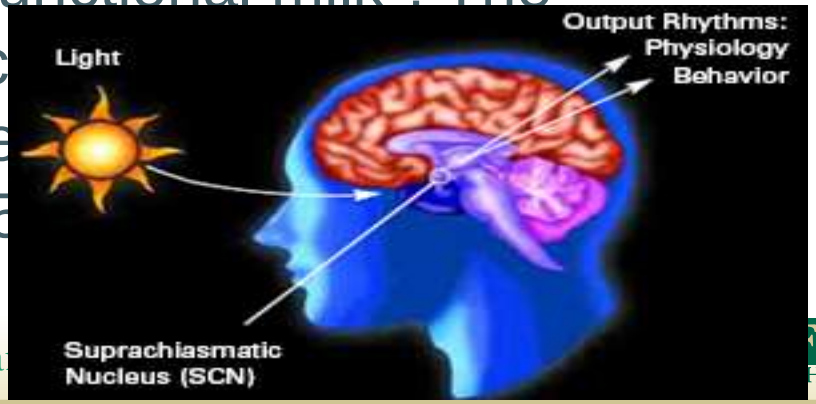
Hormonal Relationships

- Inhibition of dopamine release by melatonin has been demonstrated in specific areas of the mammalian central nervous system (hypothalamus, hippocampus, medulla-pons, and retina).



Melatonin

- Rises in sleep
- Daytime levels higher in people who have had poor sleep
- A potential timekeeper of circadian rhythm in some animals(Asher et al., "Chrono-functional milk": The difference between melatonin c
milk versus day-milk under diffe
conditions. Chronobiol Int. 2015



Melatonin

- Few studies of melatonin in human milk

Partcipants

- 45 exclusively lactating women studied at 4-6 wks postpartum
  - Mean age 28
  - Mean parity 1.8
  - Mean Income $25,000
  - 91% Caucasian

Instruments

- Profile of Mood states (POMS)
- Cohen Perceived Stress Scale
- Epworth Sleepiness Scale

Data Collection

- Hindmilk sample
- Venous blood sample
- Demographics and Instruments

- All collected in the participants' homes
- $50 honorarium

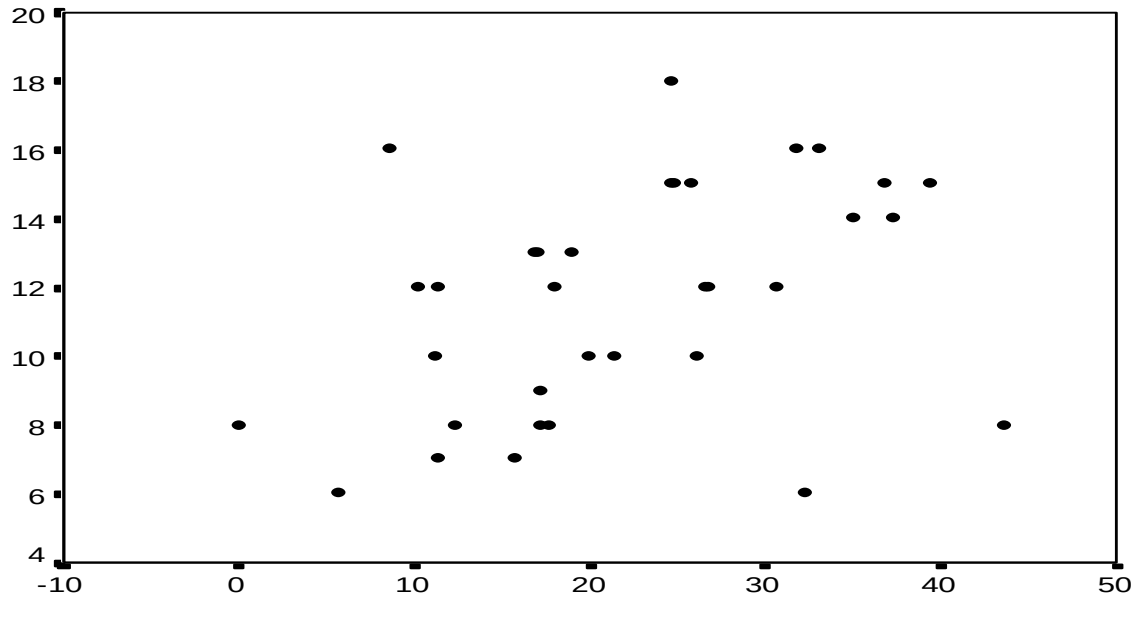
Prolactin and Melatonin

- Serum and milk prolactin levels were correlated (r=.51, p=.01)
- Lower milk prolactin was associated with higher milk melatonin (r=-.56, p=.01)
- Milk melatonin positively correlated with
  - Epworth Sleepiness scores (r=.4, p=.02) and POMS-fatigue scores (r=.44, p=.009).

Prolactin and Melatonin

- Milk Prolactin inversely correlated with Epworth Sleepiness score (r=-.49, p=.002) and POMS-fatigue scores (r=-.37, p=.05), POMS-confusion (r=-.51, p=.006); Positively correlated with milk sIgA (r=.41, p=.03).

Sleepiness and Milk Melatonin



Conclusions

- The data suggest that milk prolactin and melatonin are associated with stress and fatigue in postpartum breastfeeding mothers
- Is this a dyadic interaction?
- Can babies utilize melatonin in milk?

Study Two

Thibeau, S., D'Apolito, K., Minnick, A., Dietrich, M. Kane, B., Cooley, S., Groer, M.

Relationships of Maternal Stress with Milk Immune Components in African American Mothers of Healthy Term Infants

Breastfeeding Medicine 2016 Jan-Feb;11:6-14.

Methods

- African American mothers of a healthy term infant were given instructions to collect milk (Days 3, 9, and 14) and saliva (Day 9), as well as complete three Perceived Stress Scale questionnaires (Days 3, 9, and 14) and a survey of pregnancy stressors experiences.
- Pearson correlations and linear regressions were performed to assess the relationships of maternal stressors with milk immune comnponents.

Results

- There was at least one statistically significant correlation of a maternal stressor with nine of the 10 milk immune components (effect sizes ranging from r = 0.22 to 0.38) on Days 3 and 9. Of all milk immune variables, epidermal growth factor had the most associations with maternal stress indicators. No mediational relationship of cortisol with milk immunity was observed.

For example:

- The environmental stressor of the number of children under mothers' care was inversely correlated with MCP-1 on Day 3 and IL-6 and TNF-α on Day 9

Study Three

Baumgartel., K., Spatz, D. Groer, M. Conley, Y. (2016).

The Impact of Promoter Polymorphisms on Cytokine Concentration in Preterm Breast Milk and Subsequent Infant Outcomes. Journal of Human Lactation (in revision).

Purpose

- to examine relationships between milk Interleukin genotype and levels of Interleukins in milk from mothers of VLBWs (N=63)

Methods

- A study was conducted among mothers (n=63) who delivered very low birthweight infants (n=74, including multiples). Maternal DNA was extracted from breast milk and genotyped using TaqMan.
-

Results

- Multivariate analysis showed trending relationships between maternal IL-6 and IL-10 SNPS and levels in the milk, as well as fecal calprotectin and IVH

Study Four (pending)

Milk has a microbiome

- *microbiome in human milk*, the first food introduced into the gastrointestinal tract, and which may orchestrate, program and time the future development of the communities of microbes living in the child's gut.
- Signature gut microbiome develops early

Components of the milk microbiome

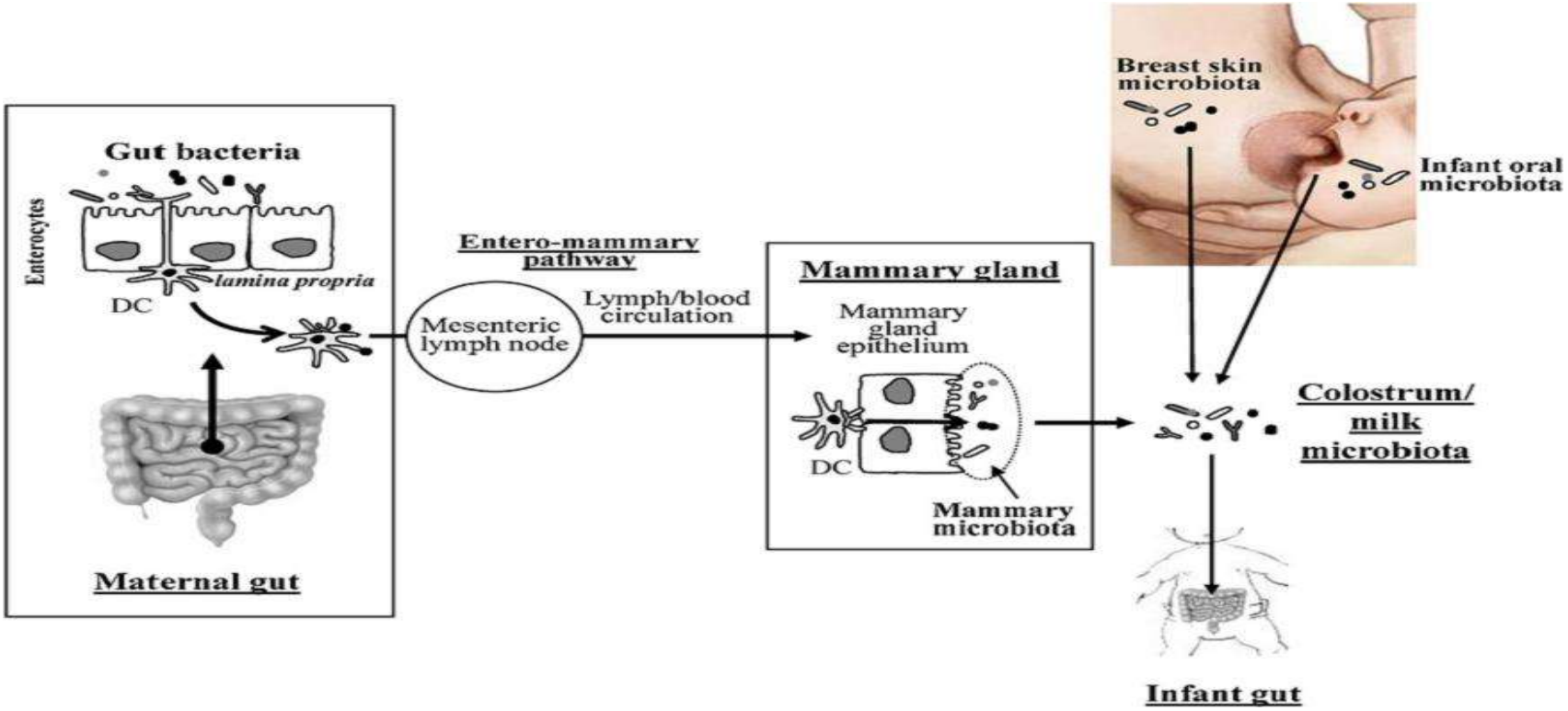
- 10^3 to 10^4 colony forming units in every ml of human milk
- most frequently cultured bacteria in human milk are: Staphylococcus, Streptococcus, Lactotoccus, Weissella, Enterococcus, Propionibacterium, Lactobacillus, and Bifidobacterium.
- NGS has identified far more diversity

Breastmilk

- “lean”-promoting microbiota : Increased Bacterioides, decreased Firmicutes

Influences

- Little known about what influences milk microbiota
  - Specific microbial genera were reported to change over the time of lactation
  - obese women had less diversity and a different microbiome compared to lean mothers
  - Microbiome changes over time and different dependent upon whether mother is exclusively or partially breastfeeding

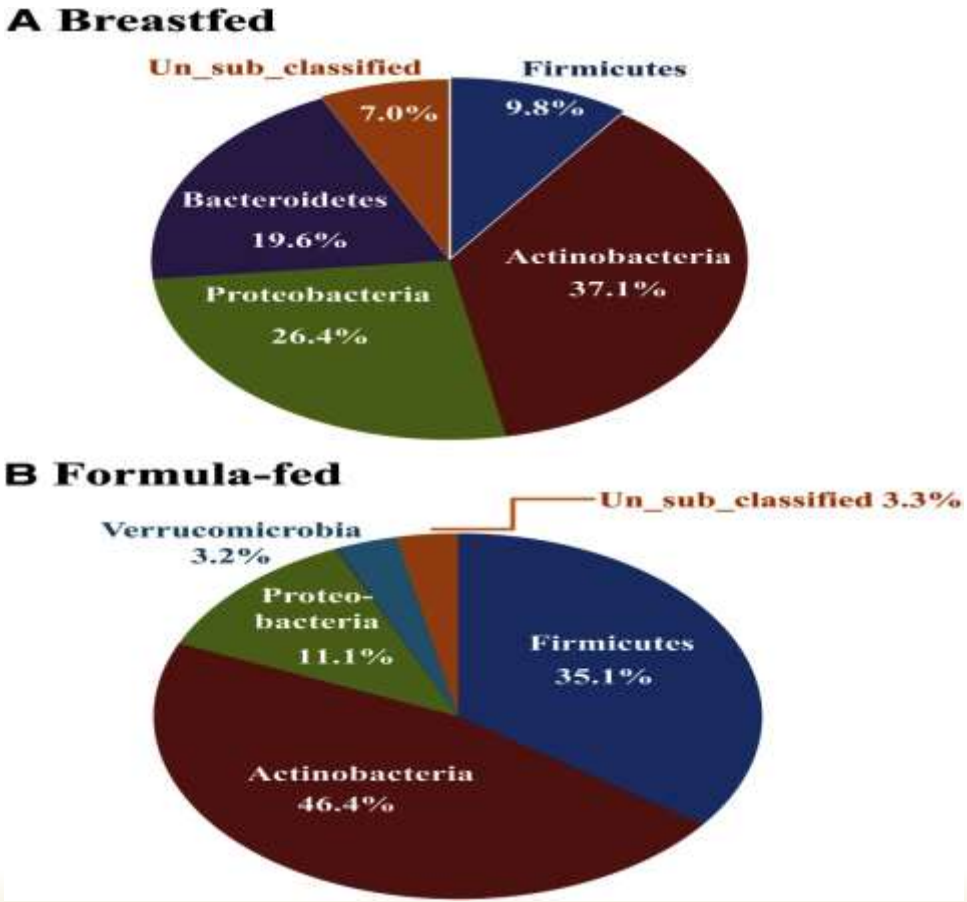

Gut bacteria
Enterocytes
lamina propria
DC
Maternal gut
Entero-mammary pathway
Mesenteric lymph node
Lymph/blood circulation
Mammary gland
Mammary gland epithelium
DC
Mammary microbiota
Breast skin microbiota
Infant oral microbiota
Colostrum/ milk microbiota
Infant gut

Source of Microbes

- Most from maternal GI tract
- One idea is that dendritic cells sample, engulf, and transport bacteria and home to the lactating breast
- Another idea is that bacteria come from infant's mouth

Milk Biochemistry

- More gut Bifidobacteria (lactobacilli) 60-90% in breastfed; ~50% in Formula fed; FF also have higher Bacteroides, Clostridia, Enterococcus, Staphylococcus
- Rich diversity of HMOs…exceeding other species by up to 100%..promote Bifidobacteria
- BF babies gut microbiota contain bacteria specialized to metabolize HMOs



Donovan et al., Adv. Nutr. 3:450S-455S, 2012

Study Five

The MOM study
R21 study funded by NIH

- Maureen Groer, Terri Ashmeade, Allyson Duffy, Shannon Morse, Bradley Kane , Shaun Cooley Ming Ji

Introduction

Human milk is recommended for preterm infants, and is protective against several neonatal illnesses. The immune composition of preterm milk (PTM) differs in several ways from term milk (TM).

Little is known about regulation of cytokines, chemokines and growth factors (CCGF) and secretory Immunoglobulin A (sIgA) in PTM.

Roles of various CCGFs include infant gut maturation, contribution to microbiome development, infant immune function, and many other undiscovered roles

Research Questions

- What is the natural course of CCGF and sIgA in human milk across a NICU stay in mother-preterm infant dyads ?
- What are relationships between maternal and infant factors and milk CCGF and sIgA?

Sample

- 76 VLBWs, mean weight 1077 Gms, GA 28 weeks; followed for 6 weeks in NICU
- PTM 0.5 ml aliquots collected daily, pooled weekly and assayed for IL-4, IL-6, IL-10, TNF- α, MCP – 1, MIP – 1^{α}, IP-10, IL-8, EGF by MagPix. Daily volumes of PDM, PTF recorded
- sIgA measured by ELISA (ALPCO)
- Fecal Calprotectin measured weekly in stool (CalPrest)

Race	
Caucasian	31.2
African American	40.3
Hispanic White	18.2
Hispanic Black	1.3
Asian/Pacific Islander	2.6
Other	6.5
Education	
Elementary school	5.2
Middle school	9.1
High school	57.2
College graduate	19.4
Post graduate	7.8
Missing	1.3
Income	
>$14,999	23.4
$5,000-$14,999	18.2
$15,000-$24,999	14.3
$25,000-$39,999	15.6
$40,000-$69,999	3.9
>$70,000	9.1
Unknown	1.3
Marital Status	
Married	37.7
Single	54.5

Sample

Table 2 Infant Characteristics

Infant Characteristics	Values (S.D.)
Infant Birth Weight (Grams) (n=74)	1077.7±219.5
Apgar at 1 minute (n=74)	6.00±1.92
Apgar at 5 minutes (n=74)	7.47±1.52
Gestational Age (n=76)	28.35±2.39
Score for Neonatal Acute Physiology-Perinatal Extension (SNAPPE -II)(n=74)	19.41±16.9
Weight at 6 weeks of age (Grams) (n=64)	1867.22±317.6
Weight at Discharge (Grams) (n=70)	2695.77±911.5

Perinatal Morbidity in the Sample

Morbidity	Number of Infants (N=77)
PFO or PDA	23 (30%)
Other Cardiac problem	8 (10.5%)
CLD	4 (5.3%)
IUGR	11 (14.5%)
ROP	14 (18.4%)
Sepsis	11 (14.5%)
IVH	9 (11.8%)
NEC	3 (3.9%)
Deaths	2 (2.6%)

PFO=patent foraman ovale PDA=patent ductus arteriosus CLD=chronic lung disease IUGR=intrauterine growth restriction ROP=retinopathy of prematurity IVH=intraventricular hemorrhage NEC=necrotizing enterocolitis

Feeding

The first 60 maternal milk samples were labeled and fed to the infant in order. Then infants were fed freshly pumped milk when available, then thawed and prepared frozen MOM. They were further supplemented with varying amounts of HMF, PDM and PTF.

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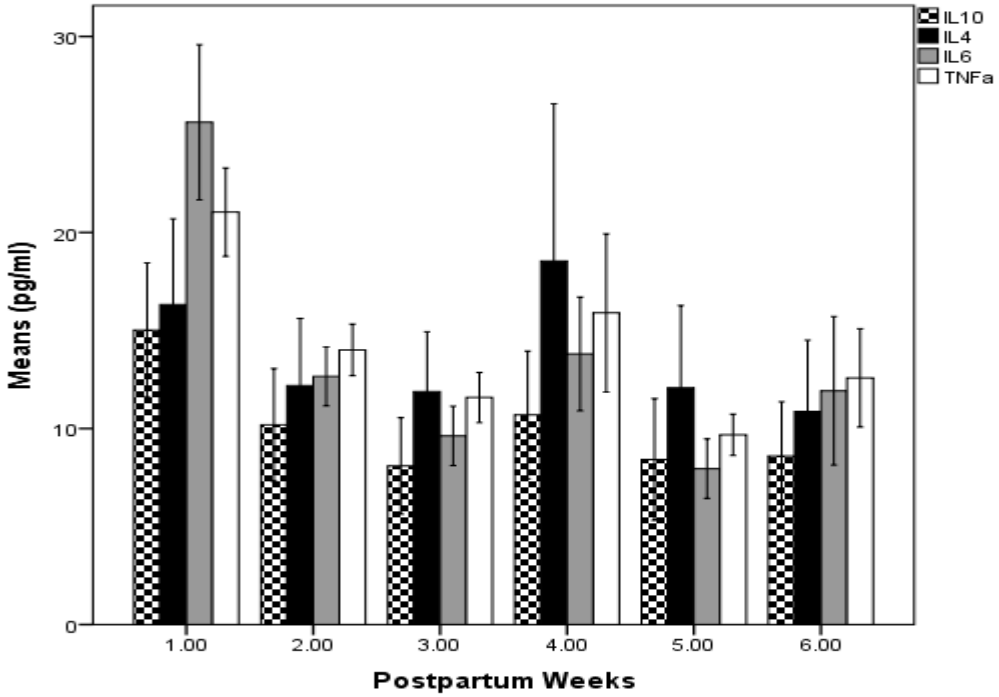
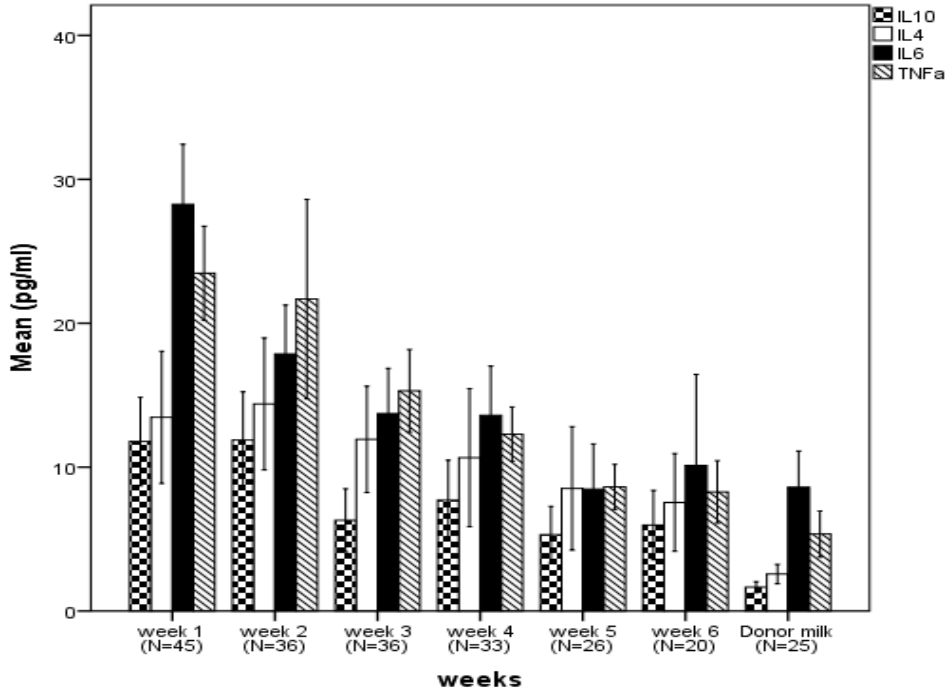
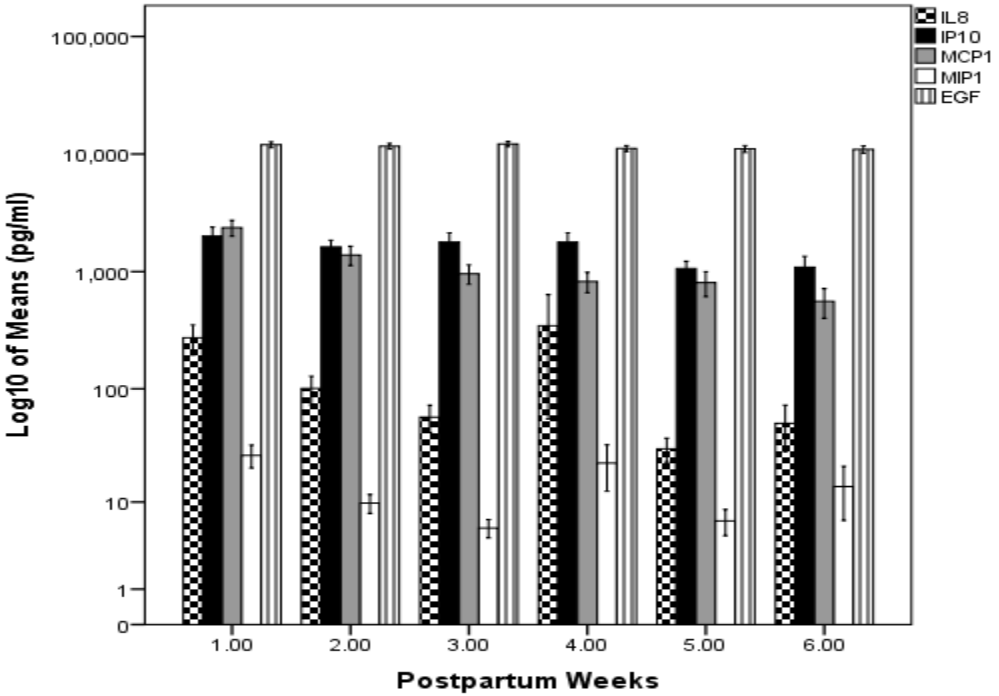
Cytokines


IL10
IL4
IL6
TNFa
Means (pg/ml)
30
20
10
0
1.00
2.00
3.00
4.00
5.00
6.00
Postpartum Weeks

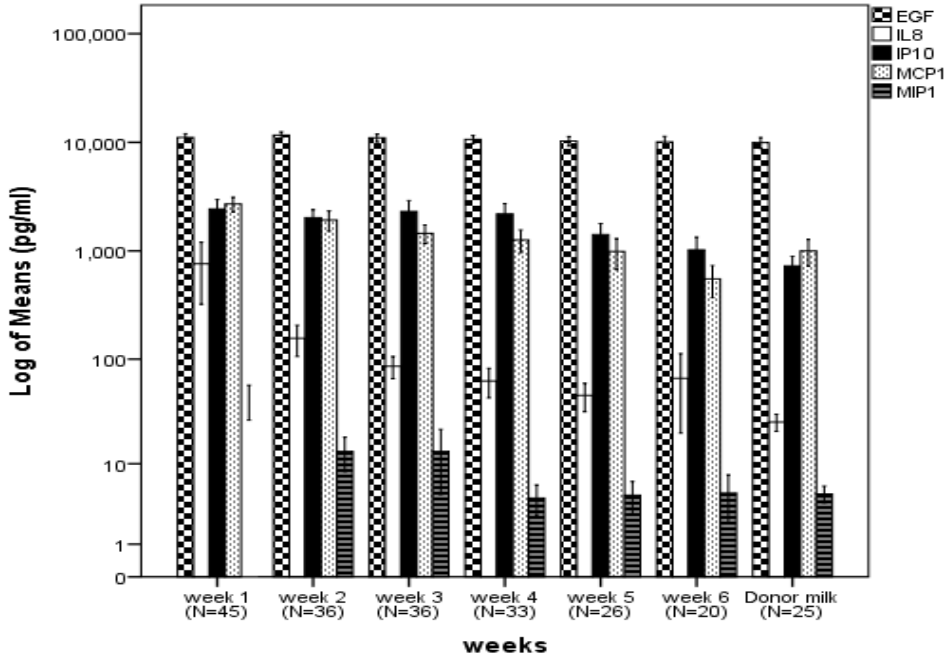
Donor Milk Cytokines



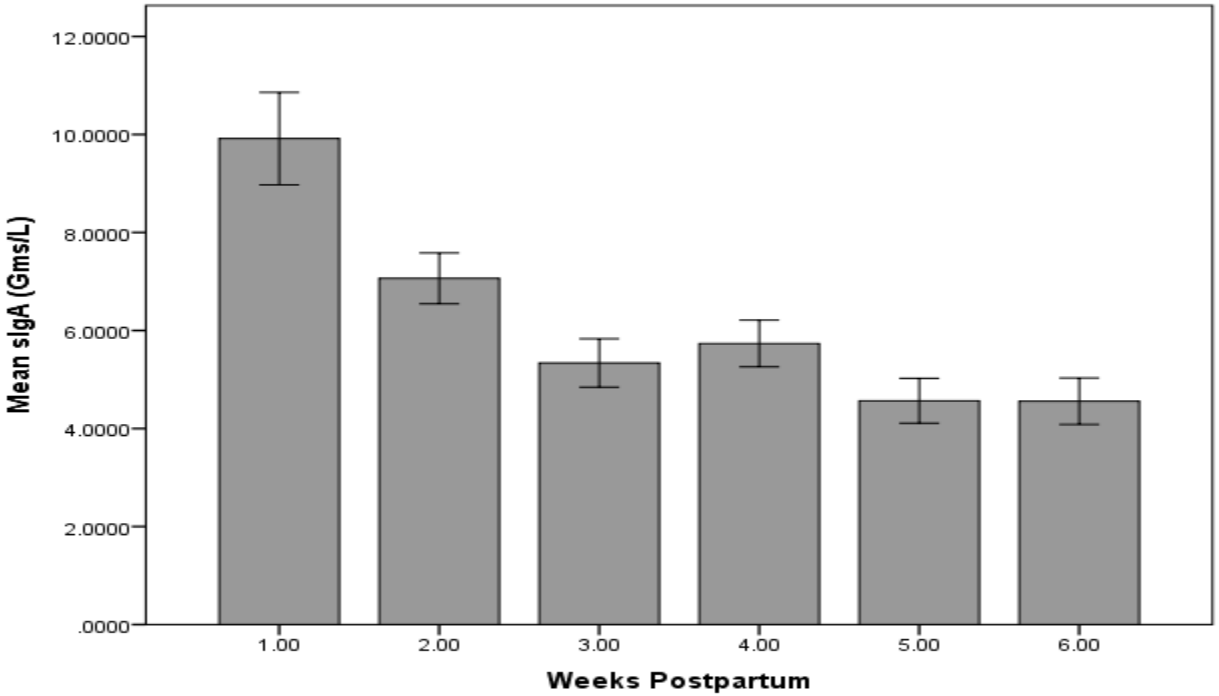
Chemokines and Growth Factors


IL8
IP10
MCP1
MIP1
EGF
100,000
10,000
1,000
100
10
1
0
Log10 of Means (pg/ml)
1.00
2.00
3.00
4.00
5.00
6.00
Postpartum Weeks

Donor Milk Chemokines and Growth Factors



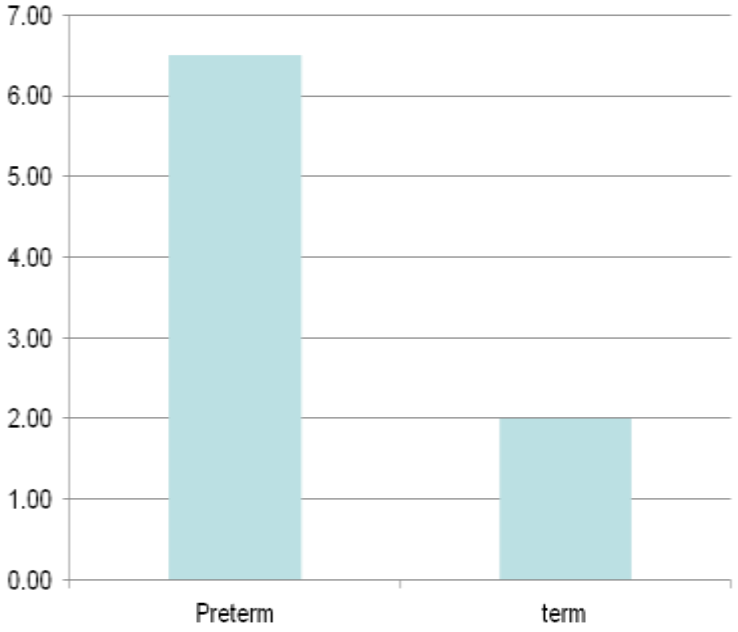
SIgA

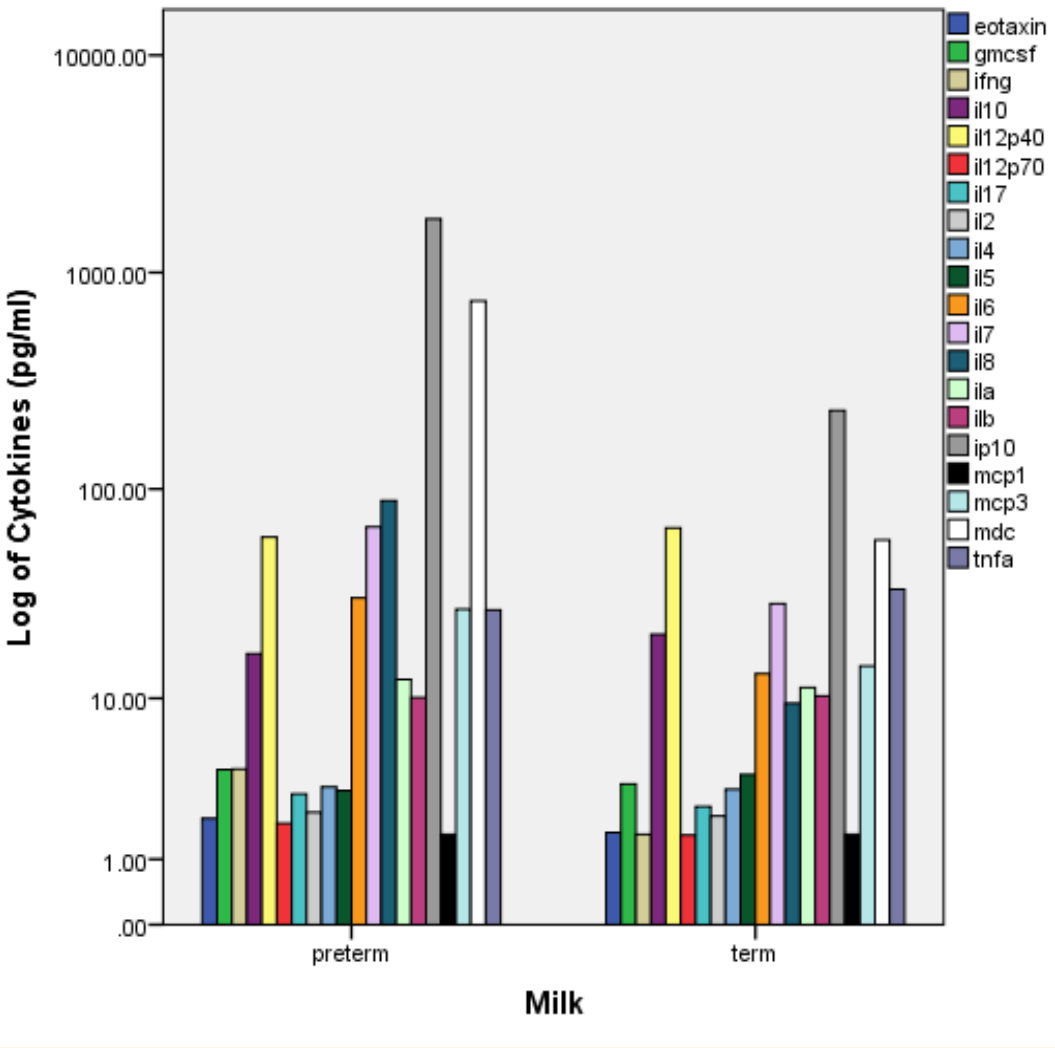

12.0000
10.0000
8.0000
6.0000
4.0000
2.0000
.0000
Mean sIgA (Gms/L)
1.00
2.00
3.00
4.00
5.00
6.00
Weeks Postpartum

Immune factors

- Secretory Immunoglobulin A is 5 times HIGHER in preterm compared to term milk
- Cytokines, Chemokines and Growth factors have different levels in preterm milk compared to term milk

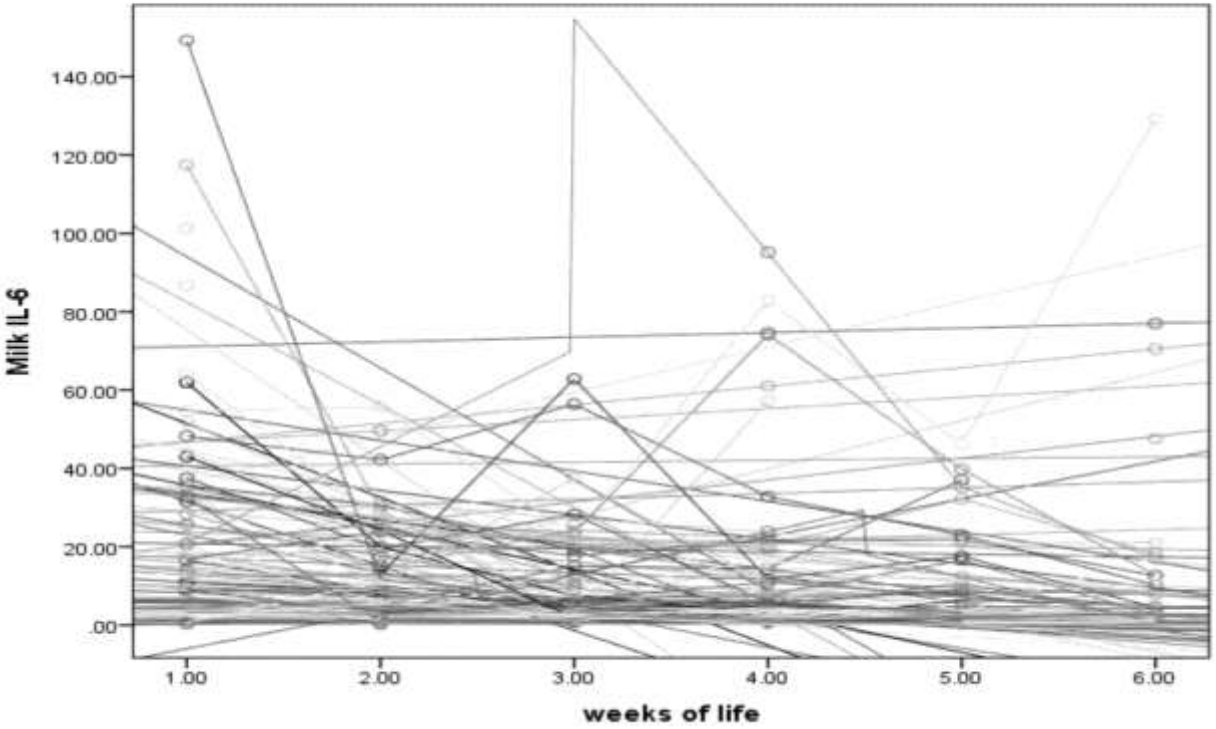
Gms/L




Log of Cytokines (pg/ml)
10000.00
1000.00
100.00
10.00
1.00
.00
preterm
term
Milk
eotaxin
gmcsf
ifng
il10
il12p40
il12p70
il17
il2
il4
il5
il6
il7
il8
ila
ilb
ip10
mcp1
mcp3
mdc
tnfa

Variability of CCGF

Figure 1


Milk IL-6
140.00
120.00
100.00
80.00
60.00
40.00
20.00
.00
1.00
2.00
3.00
4.00
5.00
6.00
weeks of life

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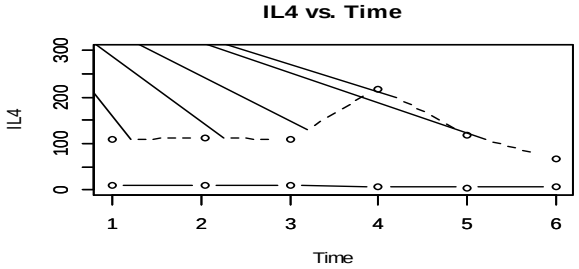
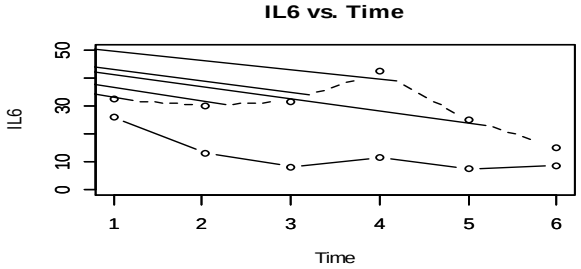
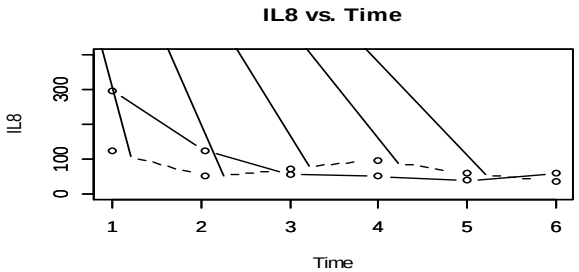
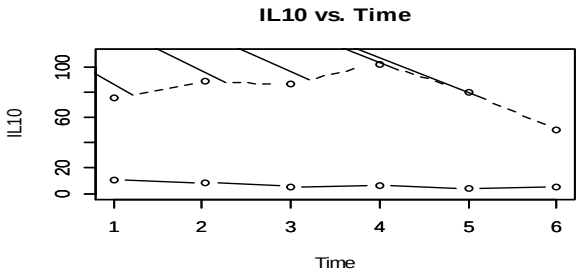
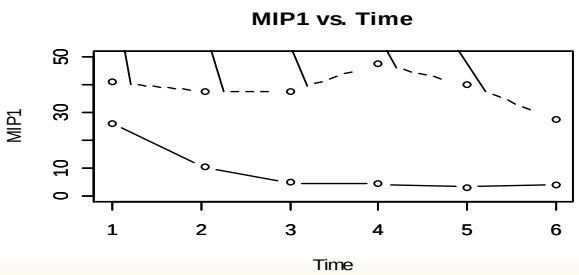
Trajectory Analysis

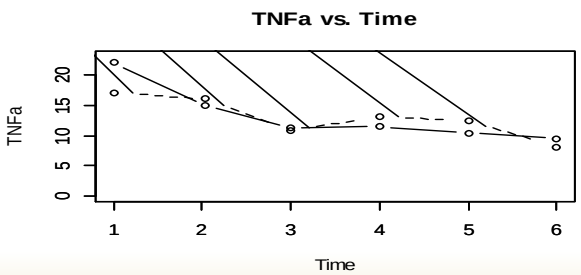

IL4 vs. Time
IL4
0
100
200
300
1
2
3
4
5
6
Time


IL6 vs. Time
IL6
0
10
30
50
1
2
3
4
5
6
Time


IL8 vs. Time
IL8
0
100
300
1
2
3
4
5
6
Time

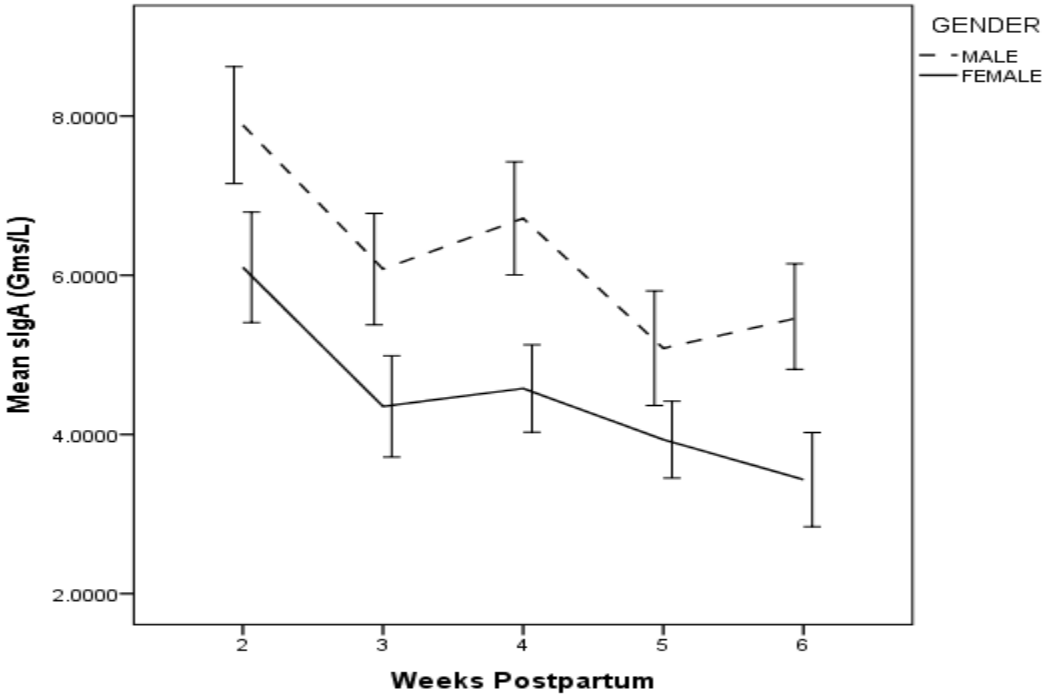

IL10 vs. Time
IL10
0
20
60
100
1
2
3
4
5
6
Time


MIP1 vs. Time
MIP1
0
10
30
50
1
2
3
4
5
6
Time

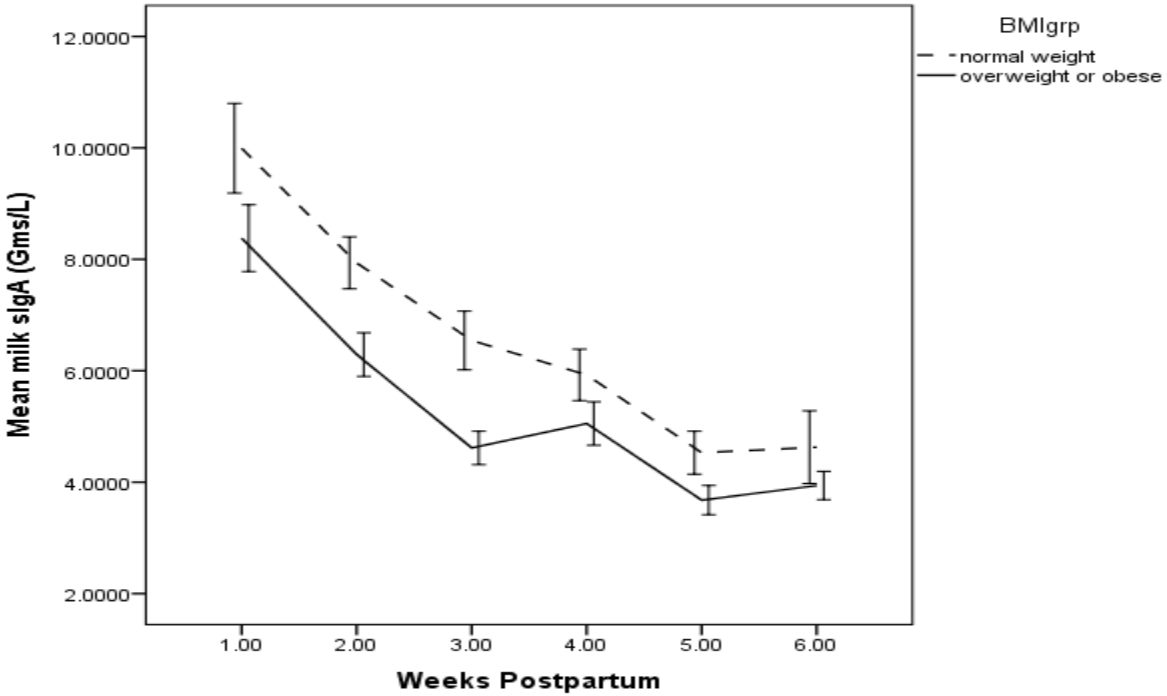

TNFa vs. Time
TNFa
0
5
10
15
20
1
2
3
4
5
6
Time

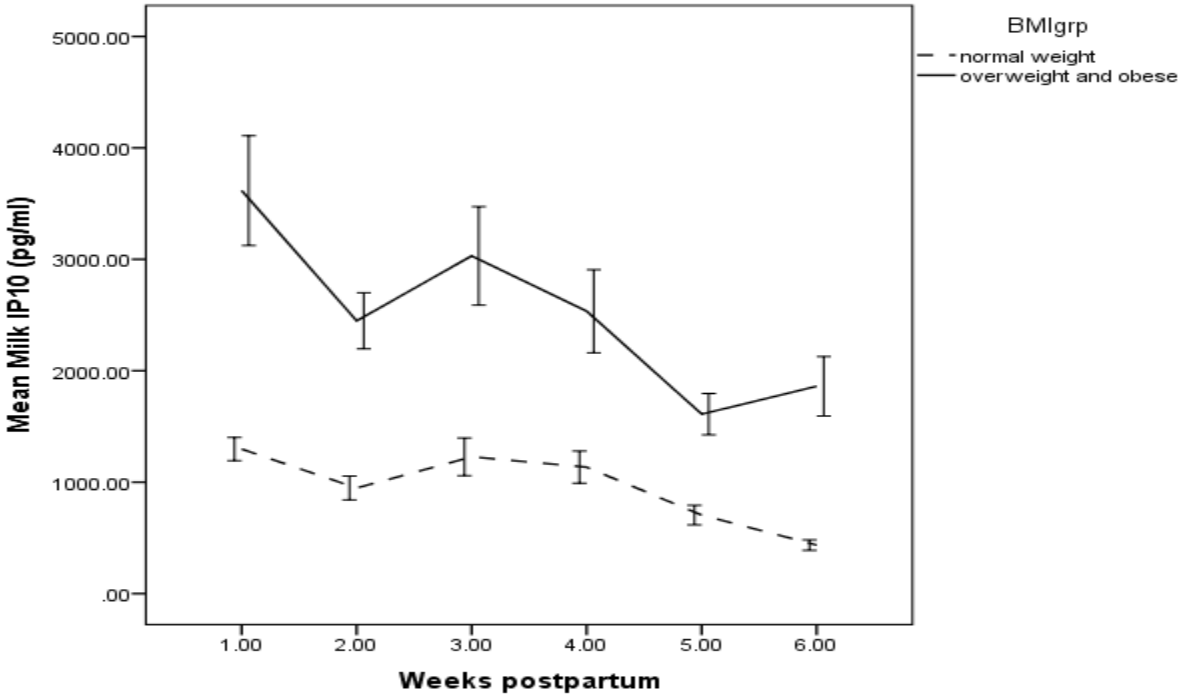
Maternal and Infant Factors

Gender


GENDER
MALE
FEMALE
8.0000
6.0000
4.0000
2.0000
Mean sIgA (Gms/L)
2
3
4
5
6
Weeks Postpartum

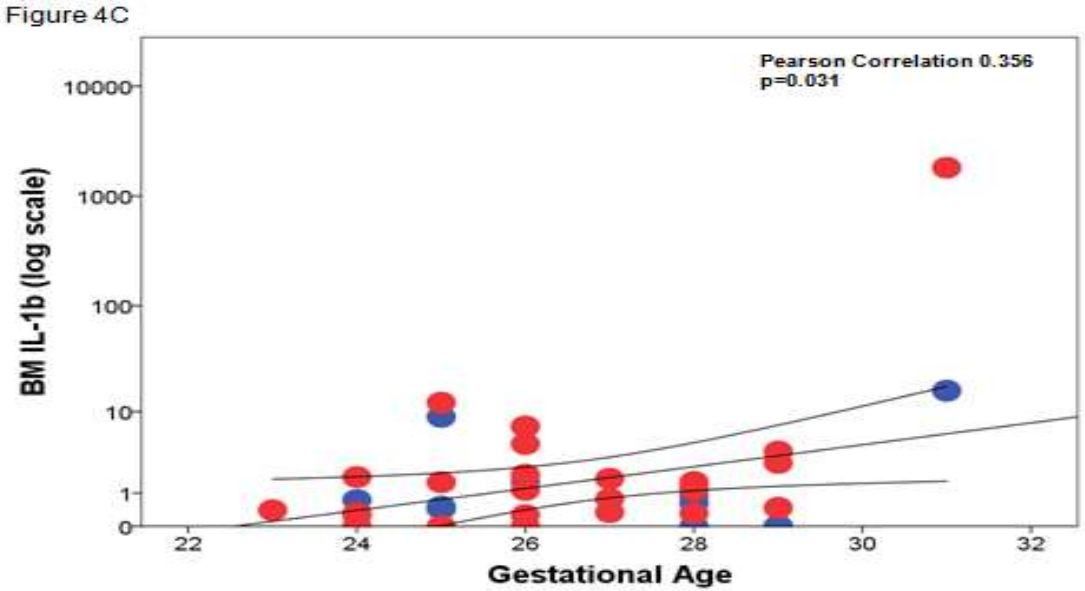
Maternal BMI


BMIgrp
normal weight
overweight or obese
Mean milk sIgA (Gms/L)
12.0000
10.0000
8.0000
6.0000
4.0000
2.0000
1.00
2.00
3.00
4.00
5.00
6.00
Weeks Postpartum


BMIgrp
normal weight
overweight and obese
Mean Milk IP10 (pg/ml)
5000.00
4000.00
3000.00
2000.00
1000.00
.00
1.00
2.00
3.00
4.00
5.00
6.00
Weeks postpartum

Study Five

High PTM cytokines in a mother with liver failure



Conclusions

Maternal and infant factors appear to play some roles in the levels of milk molecules.

- Stress
- Fatigue
- Genetics
- Illness
- Maturational stage

What we don’t know

- How critical are these relationships?
- How can normal breastfeeding be supported to support the most optimal immunity in milk?
- Should milk sharing be done if dyadic influences are critical?
- Should milk pumped in the morning be used in the morning?

Support

- Some of these projects were supported by NIH grants R01NR05000 and R21NR013094

- We are now following the stol microbiome in VLBW infants at 2 and 4 years of age in R01NR015446 to answer another critical question: Is there an influence of milk volume and immunobiology on the gut microbiome, which is now believed to be a profound influence on health and behavior.

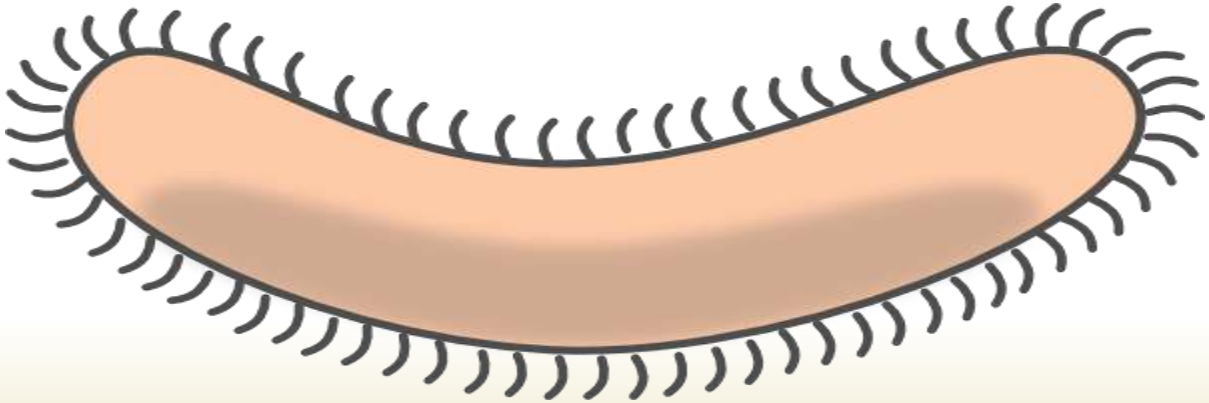
Study Six

The Preterm Infant Microbiome: Biological, Behavioral and Health Outcomes at 2 and 4 years of Age

- R01NR015446

Bacteria are Us

Bacteria have inhabited the earth for at least two and a half billion years. Our evolutionary ancestors arrived in a world dominated by microbes, and, as we evolved, so did they.

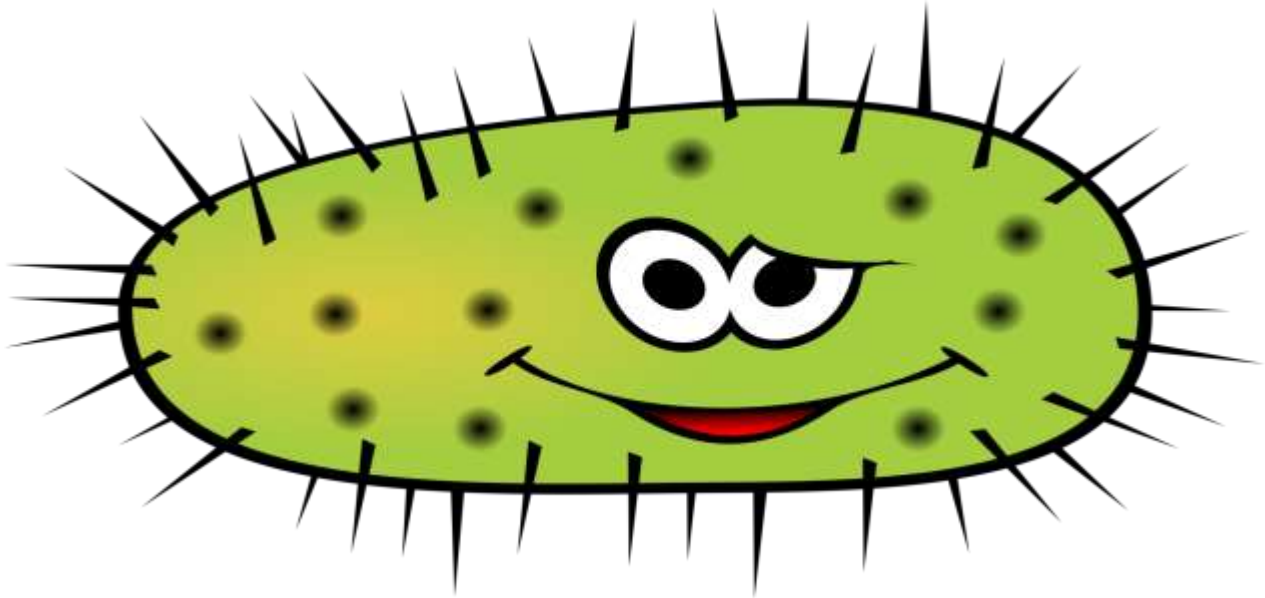


- 100 trillion beneficial microorganisms—bacteria, fungi, and viruses—populate the body and are necessary for health

Commensals vs Pathogens

- The beneficial commensals must be recognized and tolerated by the immune system
- The virulent pathogens must be attacked by the immune system
- The immune system is shaped by early life exposures to microbial life

These microbes provide the pioneer culture for the development of the commensal gut microbiome



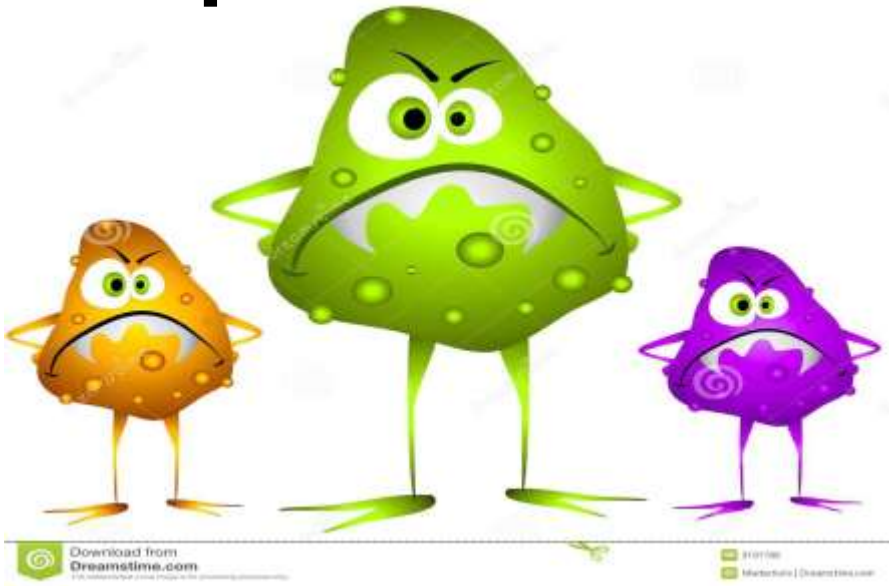
Commensals

- Contain polysaccharide-digesting enzymes that are not present in the human genome
- Dietary polysaccharides as degraded in the gut by bacteria
- Commensals inhibit growth and penetration of pathogens
- Make vitamins
- Tolerize the immune system

Other Commensal Functions

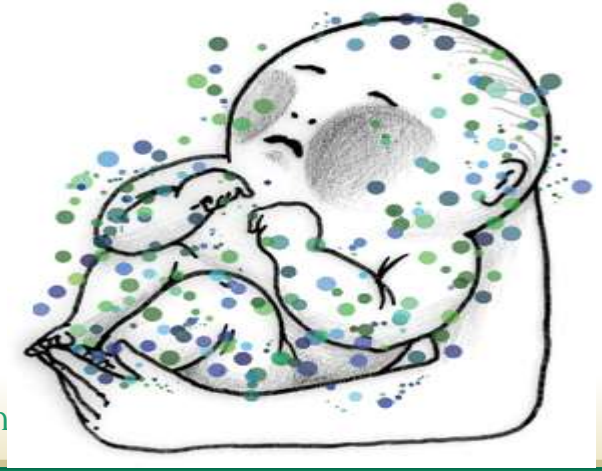
- Direct contact of bacterial cells necessary for development, regulation and response of the immune system
- Bacteria produce key metabolites that cross into bloodstream
- Bacteria produce key amino acids (eg; tryptophan) that can affect levels of serotonin and other neurotransmitters
- Bacteria have different “metabolic rates” so some are more or less efficient in using the food we eat…can result in obesity

Commensal growth inhibits ability of virulent pathogens to penetrate gut mucus and epithelium



At Birth

- Old idea was that infants were born sterile
  - Now known that meconium has a microbiota, placenta has a microbiota
    - Infant is born with a small maternally originated (largely vaginal) microbiota but within hours is heavily colonized



within hours after birth the infant is heavily colonized

The first exposures

- Maternal vaginal, enteric and skin microbes as the infant is delivered vaginally, and immediately placed on the mother's abdomen and allowed to latch
- And then: Breastmilk!

Influences on Infants' Gut Microbiota

- Caesarean section vs. vaginal birth
- Food (amount, length of breastfeeding)
- Antibiotics
- Infections
- Prenatal exposures
- Gestational age
- Genetics
- Where born

At Birth

- Vaginal birth is associated with development of a a different gut microbiome compared to C-section
  - vaginally delivered infants were found to have similar microbiota to their mother's vaginal microbiota and C-section infants harbored bacterial communities similar to skin surface microbiota
  - Current clinical trial of swabbing vaginal secretions across face of C-section newborn

C-section

- Microbes that first colonize the gut are actually SKIN microbes…and not the mother's skin

Developmental sequence

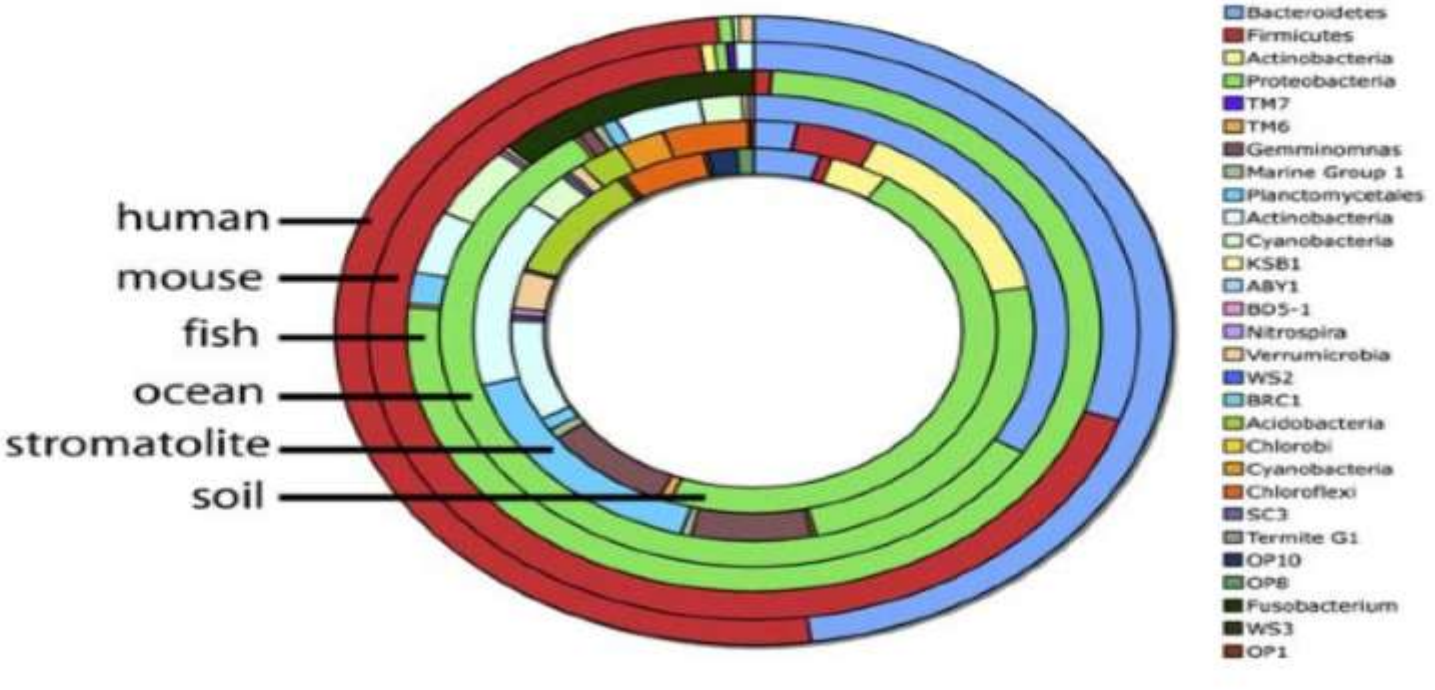
- Facultative anaerobes: 10^8-10^{10}/gram feces (Enterobacteriaceae, Enterococci, Streptococci, Staphylococci)
- Within days to week strict anaerobes (Bifidobacteria,Clostridia, Bacteroides)
- By age three signature (adult-type) microbiome

Succession

- Pioneer bacteria compete for substrate and adhesion sites (reduce high redox potential and allow anaerobes)
- Strict anaerobes flourish: Produce metabolites, signal molecules, antimicrobial compounds

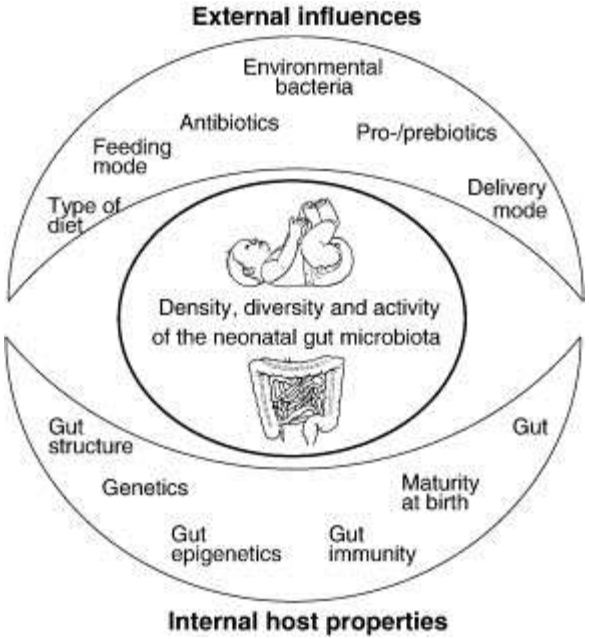
USF HEALTH COLLEGE OF NURSING
UNIVERSITY OF SOUTH FLORIDA

Nine Main Phyla in infant gut…~1000 different species



The signature microbiome (up to 3000 species)

- Most term infants achieve this around 2-3 years of age
- It remains ones “signature” for life
- Influenced by food, household, diet, antibiotics and other exposures such as pets


External influences
Environmental bacteria
Antibiotics
Pro-/prebiotics
Feeding mode
Delivery mode
Type of diet
Density, diversity and activity of the neonatal gut microbiota
Gut
Gut structure
Genetics
Maturity at birth
Gut epigenetics
Gut immunity
Internal host properties

The gut microbiome

- Enormous implications for health…and not just gastrointestinal health!
- One's signature microbiome can ultimately affect how much a person weighs, how they behave, their development, and health in many different physiological systems

How do we measure microbiome communities in milk or fecal samples?

Next generation sequencing

- Amplification of gene coding for 16SrRNA…allows for identification of thousands of different species based on diversity of this gene

Illumina MiSeq in our lab

The Preterm Infant

- <1500 Gms

ELBW and VLBW develop dysbiosis

- Inflammation
- Increased breakdown of mucus
- Increased permeability of gut epithelial tight junctions

Factors

- C-section or rapid vaginal delivery

Factors

In utero exposures (meconium microbes reflect intrauterine environment)

- Maternal infections
- Maternal antibiotics and steroids
- Maternal microbiome

Factors

- Preterm Infant Formula
- Donor Milk

Factors

- Immaturity of gut
- Immaturity of immune system

Factors

- Invasive Procedures

Factors

- Lack of maternal contact

Factors

- Universal use of antibiotics in VLBWs

Dysbiosis results

Preterm Infant Gut Microbiome

- Very abnormal: low in anaerobes, sparse, staph, enterococci, enterobacter, yeasts
- Each NICU has its own microbiota that is transferred to the infants and contains antibiotic resistant genes (Brooks et al. . Microbiome. 2014 Jan 28;2(1):1.)
- Signature microbiome does not develop at same rate as term infant

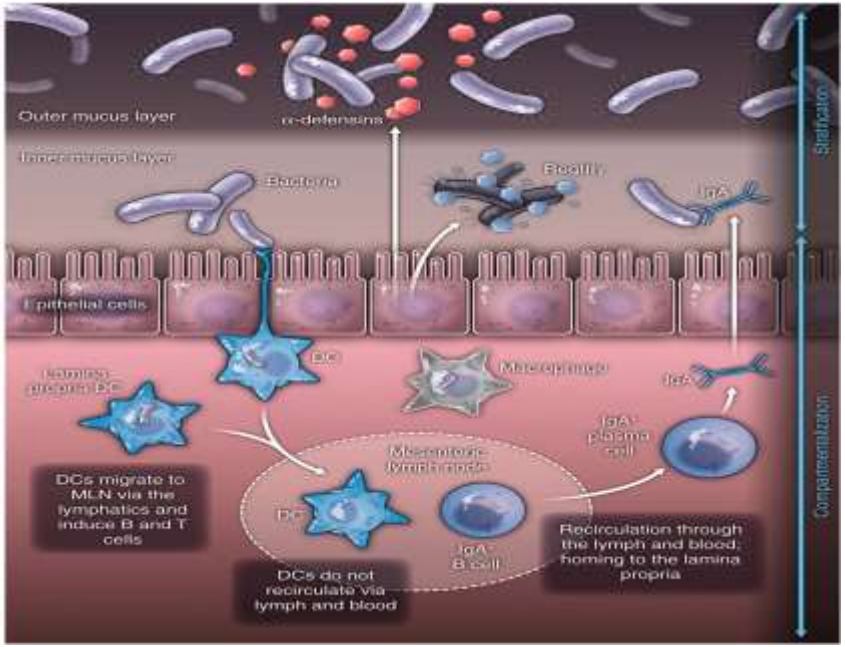
Microbiome Influences Phenotype

- Obesity
- Autoimmune disease
- Allergy
- ASD
- Depression

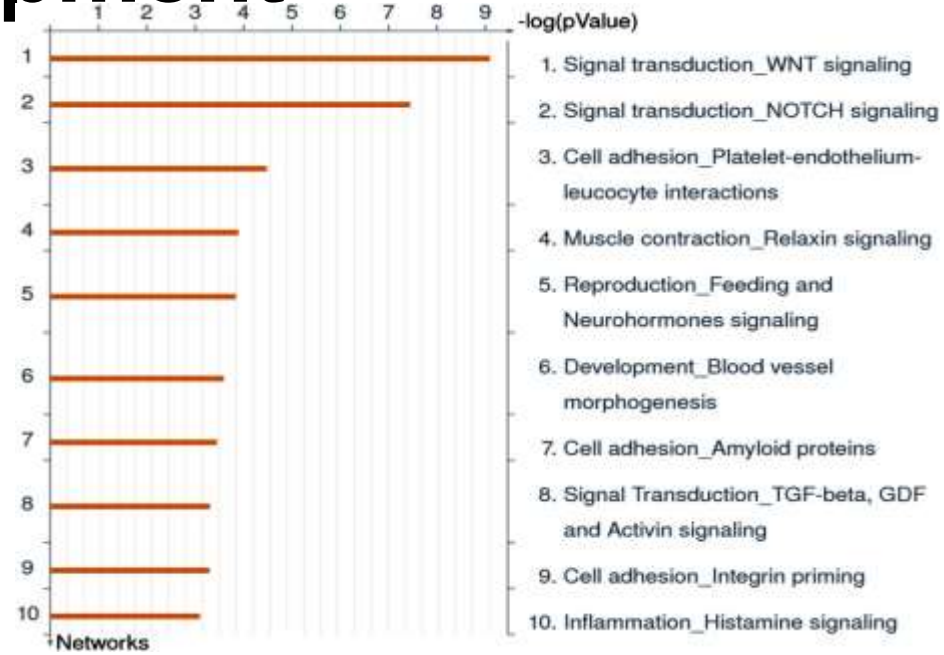
Effects of preterm infant dysbiosis

- Gut inflammation
- Sepsis
- NEC
- Catch-up growth (SGA as well as preterm); obesity and the microbiome (dysbiosis: Firmicutes/Bacteroides)
- Long term effects in VLBWs: developmental, GI, autoimmune

Gut microbiome interacts with immune system



Microbiome influences Gut Development

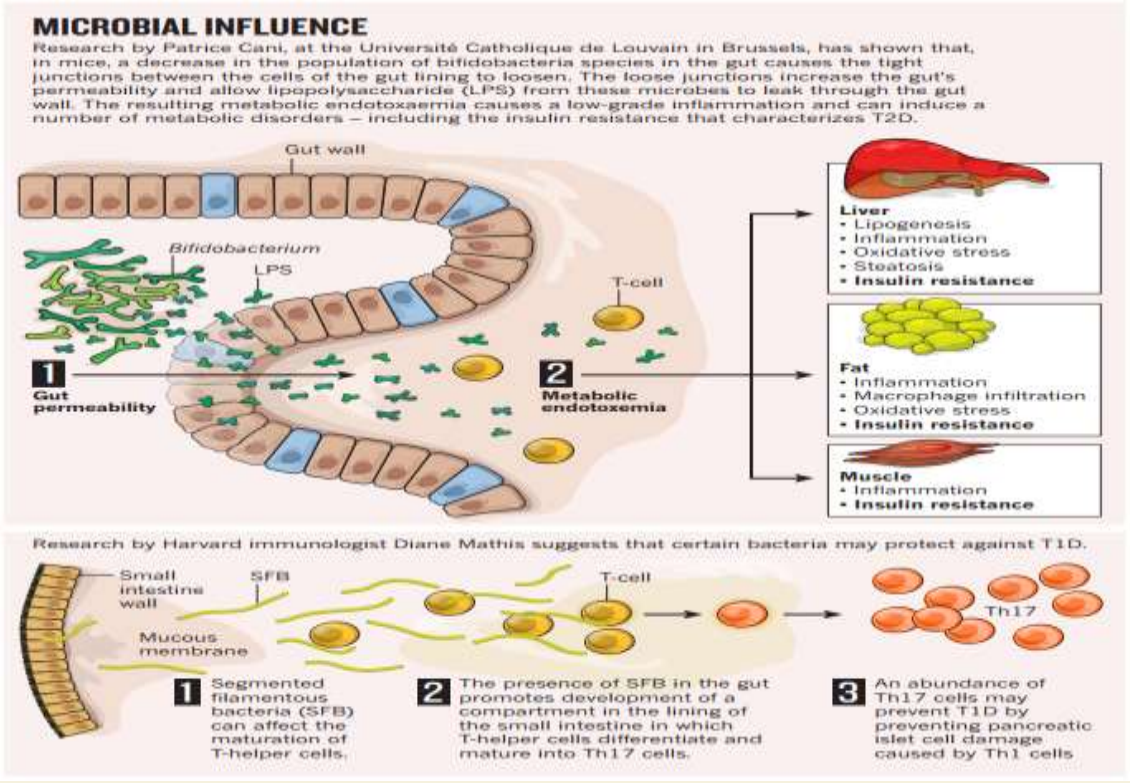


Gene networks differentially expressed in exfoliated epithelial cells from breast- and formula-fed infants. Gene expression was determined in exfoliated intestinal epithelial cells from 3-mo-old breast- and formula-

Pathogens and Leaky Gut Theory

- If dysbiosis, inflammation and leaky gut can occur
- Toxins, organism, undigested food, medications, metabolites, can leak out
- Immune response produces potential widespread effects…diabetes, asthma, lupus, multiple sclerosis, depression, anxiety, autism

Leaky Gut


MICROBIAL INFLUENCE
Research by Patrice Cani, at the Université Catholique de Louvain in Brussels, has shown that, in mice, a decrease in the population of bifidobacteria species in the gut causes the tight junctions between the cells of the gut lining to loosen. The loose junctions increase the gut's permeability and allow lipopolysaccharide (LPS) from these microbes to leak through the gut wall. The resulting metabolic endotoxaemia causes a low-grade inflammation and can induce a number of metabolic disorders – including the insulin resistance that characterizes T2D.
Gut wall
Bifidobacterium
LPS
T-cell
1 Gut permeability
2 Metabolic endotoxemia
Liver
• Lipogenesis
• Inflammation
• Oxidative stress
• Steatosis
• Insulin resistance
Fat
• Inflammation
• Macrophage infiltration
• Oxidative stress
• Insulin resistance
Muscle
• Inflammation
• Insulin resistance
Research by Harvard immunologist Diane Mathis suggests that certain bacteria may protect against T1D.
Small intestine wall
SFB
Mucous membrane
T-cell
Th17
1 Segmented filamentous bacteria (SFB) can affect the maturation of T-helper cells.
2 The presence of SFB in the gut promotes development of a compartment in the lining of the small intestine in which T-helper cells differentiate and mature into Th17 cells.
3 An abundance of Th17 cells may prevent T1D by preventing pancreatic islet cell damage caused by Th1 cells

Regulation of the microbiota-brain-gut axis is essential for maintaining homeostasis, including that of the CNS.

The germ free mouse

- A model for what happens when an organism does not have a gut microbiome

Germ free mouse incubators at NIH

Behavior in the germ free mouse

- Increased response to stress
- More daring
- Reduced anxiety
- Reduced non-spatial memory
- Altered monamines
- lack an ability to recognize other mice with whom they interact
- Altered neurotrophin levels

Microbiota can then be manipulated in germ free mice

- When colonization of the intestines of one strain of germ-free mice with bacteria taken from the intestines of another mouse strain: the recipient animals would take on aspects of the donor's personality. Naturally timid mice would become more exploratory, whereas more daring mice would become apprehensive and shy.
- These tendencies suggested that microbial interactions with the brain could induce anxiety and mood disorders.

The household microbiome (Dr. Jack Gilbert, Argonne National lab)(Science. 2014 Aug 29;345(6200):1048-52)

The Home Microbiome Project followed seven families, which included 18 people, 3 dogs and 1 cat, over the course of 6 weeks. The participants in the study swabbed their hands, feet and noses daily to collect a sample of the microbial populations living in and on them. They also sampled surfaces in the house, including doorknobs, light switches, floors and countertops.

Home is where the microbes are

- They found that people substantially affected the microbial communities in a house—when three of the families moved, it took less than a day for the new house to look just like the old one, microbially speaking.

The “home” of the preterm infant for many weeks

Behavior

- Microbiota NEED us to be social
- Gut-Brain axis involved in behavior

Microbiome and ASD

- women who suffer from a high, prolonged fever during pregnancy are up to seven times more likely to have a child with autism.
- 40 to 90 percent of all children with autism suffer from gastrointestinal symptoms
- Dysbiosis has been noted in gut
- Neuroinflammation
- Study of vancomycin Rx reversing Sx

New meaning to “gut feelings”