Liquid Gold: Immunological and Nutritional Factors in Breast milk

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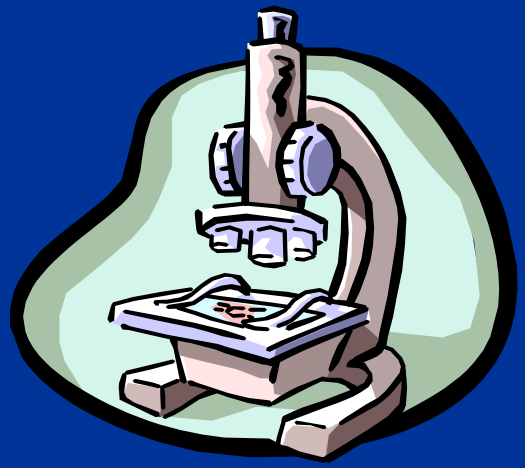


Immunological Factors

Protective Role of Breastfeeding

Protection Against Infections

- Intestinal infections
  - In poor countries the risk of dying from diarrhea for non-breastfed infants is 25 times that of the exclusively breast-fed
  - Breastfeeding gives protection against diarrhea caused by:
    - *Vibrio cholerae*
    - *E.coli*
    - *Campylobacter*
    - *Shigella*
    - *Giardia lamblia*

Protection Against Infections

- Respiratory tract infections
- Otitis media
- Botulism
- Necrotizing enterocolitis
- Urinary tract infections
- Neonatal septicemia
- Pneumonia

Protection Against Infections

- WHO estimates that an increase in breastfeeding by 40% world-wide would reduce:
  - diarrhea deaths by 66%
  - deaths from respiratory infection by 50%
- In children under the age of 18 months

Protection Against Infections after Weaning

- Protection against *Haemophilus influenzae* type b (Hib) infection is enhanced 10 years after lactation
- For each week of breastfeeding the protection improved
- Breastfeeding beyond 13 weeks provides prolonged protection against diarrhea even when solid foods have been introduced in the meantime

Protection Against Infections after Weaning

- Children who have been exclusively breastfed without solid foods being introduced remain better protected against respiratory infections for 7 years
- Breastfeeding for >3-4 months decreases the risk of otitis media up to the age of 3 years
- Non-allergic bronchitis decreased for up to 6-7 years after termination of breastfeeding
  - The effect is enhanced for every additional week of breastfeeding

Immunological Protection

- Agents in human milk:
  - Provide passive protection of the infant against infection during lactation
    - Mother's system provides the protective factors
  - Stimulate the immune system of the baby to provide active protection
    - Infant's own system makes the protective factors
- The effects may last long after weaning

Protective Factors Provided by Breastfeeding

- Transmission of fewer pathogens
  - Breast milk is sterile
- Contains preformed antimicrobial agents:
  - Antigen-specific (e.g. antibodies)
  - Non-specific (e.g. lysozyme)
- Other antimicrobial agents are formed as human milk components are broken down during digestion

Protective Factors Provided by Breastfeeding

- Protect by non-inflammatory mechanisms
- Stimulate maturation of the infant's immune system
- Promote establishment of a protective microbial population in the infant's digestive tract

Characteristics of Protective Agents in Human Milk

- Persist throughout lactation
- Resist digestion in the infant's digestive tract
- Protect by non-inflammatory mechanisms
- Are the same as at mucosal sites (e.g. in the lining of the digestive tract)

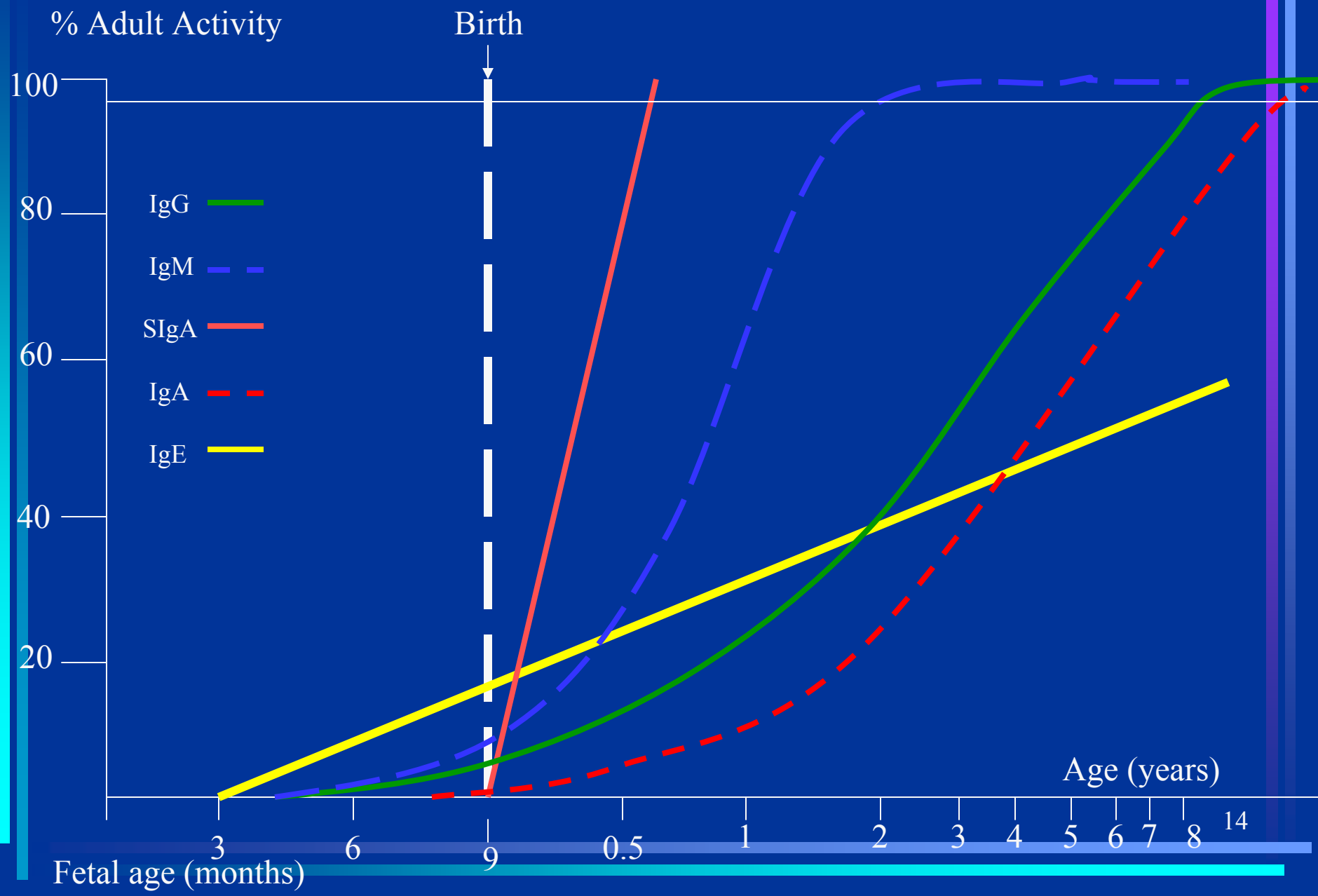
Immune System of the Normal Neonate

- Is immature
- Major elements of the immune system are in place
- But do not function at a level to provide adequate protection against infection
- The level of immunoglobulins (except maternal IgG) is a fraction of that of the adult

Immune System of the Normal Neonate

- Phagocytes can engulf foreign particles
- But their killing capacity is negligible during the first 24 hours of life
- The function of the lymphocytes is not fully developed

Development of Immunocompetence with Age



Immunoglobulins (Antibodies)
IgG

- IgG is the only antibody transported across the placenta to protect the fetus in utero
- IgG is produced by the mother's immune system
- Reflects the exposure of the mother to potentially pathogenic antigens

Immunoglobulins: IgG

- Provides protection of the infant for several months after birth
- Is passive protection
- Maternal IgG is gradually removed from the infant's circulation
- Infant produces its own IgG starting immediately after birth:
  - This is active protection

Immunoglobulins: IgG

- In humans there is minimal transportation of IgG to external secretions such as milk
- Human milk contains very little IgG
- IgG provides very little protection to the intestinal tract of the newborn

Immunoglobulins: Secretory IgA (sIgA)

- Antibodies in human milk are predominantly secretory IgA
- They reflect mother's immune response to foreign antigens which encounter her body via mucous membranes
- Provide protection against potential pathogens in the environment
- Under "natural conditions" this is also the environment of the infant

Immunoglobulins: sIgA

- After birth the infant's digestive tract is suddenly exposed to an onslaught of microorganisms and foreign macromolecules
- sIgA provides a linkage between the intestinal immune systems of the mother and infant
- Provides built-in protection for the immunologically naïve infant
- Is extremely important protection in areas of the world with poor sanitation

Immunoglobulins: sIgA

- sIgA antibodies pass into the infant's digestive tract in mother's milk
- Protect the lining of the infant's digestive tract
- Are not absorbed into the infant's circulation
- Resist digestion by the infant's digestive enzymes

Sequence of sIgA Production

- Mother's T-cell lymphocytes recognize foreign antigens at the mucosal surfaces of her lungs and digestive tract
- Activate B-cell lymphocytes
- Activated B-cells migrate from the bronchus or digestive tract to mammary glands
- Localize in the subepithelial cells

Sequence of sIgA Production

- B cells mature into IgA-producing plasma cells
- Plasma cells produce IgA molecules
- IgA molecules combine in pairs (dimers) joined by a peptide J-chain (joining chain)

Sequence of sIgA Production

- Dimeric IgA combines with a receptor on the basolateral membrane of the epithelial cell
- IgA and the receptor molecule proceed through the epithelium
- The receptor molecule is known as the *secretory piece*
- The assembled complex is secreted into milk

Protective Action of sIgA

- Secretory piece protects the antibody from the action of digestive enzymes
- sIgA remains immunologically active throughout the length of the infant's digestive tract
- Protects the infant from foreign antigens encountered by mother
- As long as mother and infant are together, infant is protected from pathogens in its environment

sIgA Production

- The whole sequence is controlled by hormones produced late in pregnancy and during lactation
- Is mediated by T helper cells and cytokines
- Considerable quantities of sIgA are ingested by the breast-fed infant especially during the first month

sIgA

- sIgA accounts for >90% of the immunoglobulins in human colostrum and milk
- Neonate has no sIgA at birth
- Infant commences its own sIgA production at birth
- 100% of the adult level of sIgA is normally achieved by 6 months
- Cow's milk and infant formulas are devoid of sIgA

Lactoferrin

- Predominant human whey glycoprotein
- Binds iron: each molecule has two iron-binding sites
- 80% of the iron binding sites are unsaturated
- Combines with any iron molecules in the digestive environment
- May assist in the transport of dietary iron

Lactoferrin

- Competes with iron-requiring bacteria in the digestive tract
- Reduces growth of these bacteria
- Persists along the length of the infant's digestive tract
- Some lactoferrin may be synthesized by the infant's own digestive mucosa

Lysozyme

- Enzyme (N-acetylmuramide glyconohydralase)
- Present at a high level in external body secretions (saliva, tears, milk)
- Attacks bacterial cell wall and splits it apart
- Hydrolyses ß-1,4 linkages between N-acetylmuramic acid and 2-acetyl-amino-2-deoxy-D-glucose residues

Lysozyme

- Large quantities in colostrum and milk
- Relatively resistant to digestion with trypsin or hydrolysis by gastric acid
- Persists along the length of the infant's digestive tract
- Can be detected, and is active, in feces of breast-fed infant

Comparison of Protective Agents in Human and Cow's Milk (mg in 100 mL)

Protective Factors	Human	Cow
Secretory IgA	100	3
IgG	1	60
IgM	1	30
Lactoferrin	150	Trace
Lysozyme	50	0.01

Protective agents in milk of well-nourished and under-nourished women (mg per 100mL)

1. Colostrum (1-5 days post-partum)

Group	sIgA	Lysozyme	Lactoferrin
Well-nourished	336	14.2	420
Undernourished	375	16.4	520

Protective agents in milk of well-nourished and under-nourished women (mg per 100mL)

2. Mature milk (1-6 months)

Group	sIgA	Lysozyme	Lactoferrin
Well-nourished	120	24.8	250
Under-nourished	118	23.3	270

Leukocytes in Human Milk

- Highest level in the early phase of lactation
- Gradually decline over the next 2-3 months
- Mostly neutrophils and macrophages
- Macrophages in milk are much more active than those in blood

Leukocytes in Human Milk

- Macrophages produce toxic oxygen radicals
- Increases the rate of killing compared to that of blood macrophages
- Lymphocytes generate cytokines such as interferon-γ, which aid in destroying bacteria and viruses
- Numerous other leukocyte-derived cytokines and chemokines aid in protecting the infant from infection

Oligosaccharide Glycoconjugates

- In order to colonize the digestive tract, bacteria combine with receptors on the epithelium
- Glucoconjugates combine with these receptors and block bacterial adherence
- Prevention of adherence prevents bacterial localization and multiplication

Oligosaccharide Glycoconjugates

- Examples:
  - Fucose units combine with receptors that can complex with toxins of *E.coli* and *Vibrio cholerae*
  - Mannose units combine with receptors that can complex with certain vibrios
  - Glycoproteins and glycolipids combine with receptors that complex with fimbriae of *E.coli*
  - Others prevent binding of *Streptococcus pneumoniae* and *Haemophilus influenzae*

Other Adherence-Associated Agents

- Lactadherin has recently been identified in human milk
- Is 46kDa mucin-associated glycoprotein
- Binds to rotavirus and inhibits its replication
- Reduces severity of infection, leading to fewer diarrhea-associated deaths

Fibronectin

- Protein in human milk that facilitates the uptake of particles by phagocytes
- Present in colostrum at a level of 13.4 mg per litre

Nucleosides and Nucleotides

- Are the purine and pyrimidine components of DNA and RNA
- May play a role in infant development
- No nucleosides occur in bovine milk or milk-based infant formula
- Three kinds of nucleosides occur in human milk

Nucleosides and Nucleotides

- Bovine milk is considerably lower in nucleotides than human milk
- Nucleotides increase infant's ability to produce antibodies, especially IgG
- Nucleotide supplementation led to:
  - Higher response to Hib vaccination
  - Higher response tp diphtheria vaccination

  At 7 months of age but no difference at 12 months
- Lack of nucleotides associated with increased risk of bacterial and fungal infections

Agents from Partial Digestion of Milk

- Action of lipase enzymes on lipids in milk produce fatty acids and monoglycerides
- Disrupt the lipid outer coating of enveloped viruses
- Include coronaviruses
- Also protect against *Giardia lamblia*

Agents from Partial Digestion of Milk

- ß-casomorphins are produced from human κ-casein
- Have an opioid effect
- Also act as immuno-stimulants, aiding in the immunological protection of the infant

Immunostimulants

- sIgA
  - Human milk components stimulate the infant's immune system to produce sIgA
- Interferon-γ
  - There is very little interferon-γ in human milk
  - The level of interferon-γ is much higher in the breast-fed compared to the formula-fed infant in response to a viral infection
  - Indicates that a factor in human milk is likely to have stimulated the infant's immune system to respond at a more mature level

Immunostimulants

- Fibronectin
  - Higher in the plasma of breast-fed infants than in milk itself
  - Indicates stimulation of the infant's own system
- Monocytes
  - Become much more active when incubated in human milk than in blood
  - Suggests stimulation by e.g. cytokines in milk

Immunostimulation

- Enhanced vaccine response in breastfed compared to non-breastfed infants seen in response to:
  - Hib vaccine
  - Poliovirus vaccine
  - Tetanus toxoid
  - Diphtheria toxoid
- Increased interferon-γ after measles-mumps-rubella vaccination
- Stronger T-cell response to BCG (anti-tuberculosis) vaccine

Further Evidence of Immunostimulation

- Compared to formula-fed infants, breast-fed infants show a lower incidence of:
  - lymphomas
  - juvenile-onset diabetes
  - Crohn's disease
  - allergy

Promotion of Protective Microbial Flora

- Oligosaccharides in human milk promote the growth of lactobacilli and bifidobacteria in the infant's large bowel
- Acids produced by these bacteria inhibit the growth of potential pathogens such as *E.coli, Salmonella, Shigella*
- The acidic environment is enhanced by the low buffering capacity of human milk

Promotion of Protective Microbial Flora

- Infant formulae provide a more alkaline environment
- Allows proliferation of potentially pathogenic, faster-growing microorganisms
- Studies indicate that formula-fed babies given live lactobacilli in their feed have greater weight gain than those not given the bacteria

Other Bioactive Agents in Breast milk

- There are at least 45 classes of different bioactive agents in human milk
- In addition to antimicrobial factors these include:
  - Enzymes
  - Hormones
  - Growth Factors
  - Anti-inflammatory agents

Other Bioactive Agents in Breast milk

- Examples of these agents:
  - Hormones:
    - Thyroid hormones
    - Cortisol
    - Progesterone
    - Pregnanediol
  - Research evidence suggests that a number of hormones in human milk may contribute to the maturation of the infant's digestive tract

Other Bioactive Agents in Breast milk

- Growth factors:
  - Erythropoietin
  - Human growth hormone (hHG)
  - Gonadotropin-releasing hormone
  - Epidermal growth factor
  - Insulin
  - Insulin-like growth factor-I
  - Nerve growth factor
  - Transforming growth factor-α
  - Gastrointestinal regulatory peptides
  - Thyroid-parathyroid hormones

Conclusion

- Human milk plays an irreplaceable role in infant nutrition, immunological protection and developmental effects
- In addition, breast-feeding is unique for its mode of feeding and provides an important advantage in hygiene as well as physical and emotional bonding of mother and child