REVIEW ARTICLE

Immunomodulatory Effects of Human Milk upon Immune Functions in Infants

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ABSTRACT Human milk is capable to modulate the immune system in infants in several ways. Immunorestoration is mostly dominated by immunoglobulins content of human milks especially secretary IgA, although IgG antibody also plays important role. Nucleotide content of human milk is among substances widely investigated all over the world, capable to potentiate immune response in infants by increasing NK cell activity and production of IL-2. Immunopotentiation also the result of cell content activity of human milk, interferon, and complement. Special feature of anti inflamatory property of human milk is its capability to prevent of allergic disease in infants. This because of protein in breast milk is species specific, instead the role of secretary IgA in preventing absorption of foreign macromolecules. Other substances also play an important role in the antiinflammatory properties of human milk and interestingly the paucity of initiators and mediators in human milk. **[Paediatr Indones 1999; 39:243-250]**

Introduction

There are many studies that indicate the beneficial effect of human milk feeding for mother herself and their suckling infant. For the mother it has long been acknowledged that breast-feeding increases level of oxytocin, resulting in less post partum bleeding and more rapid uterine involution, one of many advantages for the mothers. For the infant, epidemiologic research shows that human milk and breast feeding of infant provide advantages with regard to general health, growth and development, while significantly decreasing risk for large number of acute and chronic diseases.

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Evidence begin to accumulate that breastfeeding decreases incidence and severity of diarrhea,[1] lower respiratory infections,[2] bacterial meningitis,[3] urinary tract infection,[4] otitis media,[5] necrotizing enterocolitis,[6] and possible protective effect against allergic disease.[7] The protective effect of human milk upon the sucking infant is offered by some factors include pathogen specific IgA,[8] oligosaccharide,[9] glycolipid,[10] mucin,[11] and nucleotide.[12] Many studies have reported that human milk enhances development of immune system in breastfed infant compared with formula fed infant.[13,14] Modulation of immune response by specific components of human milk convincingly is of potential benefit to breastfed infants, led us endeavor to review this topic.

Immunorestorative Effect of Human Milk

Human milk processes some substances capable to restore the immune competence of the infants. The biologic activities of these substances have been demonstrated against adhesion of enteropathogenic bacteria,[15] against binding pathogens adhering to other organs and epithelial cells.[17] The immunorestorative effect may be specific or not specific. Substances play a role in the immunorestoration may be antibodies, prostaglandin, oligosacharides, fatty acids and nucleotides.

Immunorestoration by immunoglobulins

Study of Guillermo et al indicated that diarrhea attack rate caused by Campilobacter jejuni for non breastfed infants was significantly greater than that in breastfed infants. Secretory IgA milk antibody titters against glycine acid - extractable antigen of C jejuni were high in colostrum, decreased during the first month of breast feeding and generally persisted throughout lactation. Human milk consumed by children in whom Campylobacter diarrhea developed did not contain S IgA antibody to glycine acid extractable common antigen of Campylobacter.[18] S IgA in human milk also protect infant against otitis media although other factors also support the protection.[19]

In a prospective community based study of healthy breastfed infants to determine the protective effect of anti Shigella S IgA antibodies in human milk, S IgA antibodies against lipopolysaccharides of *S flexneri*, *S boydii* serotype 2, *S sonnei* and virulence plasmid associated antigens were evaluated. The author concluded that human milk protect infants against symptomatic Shigella infection when it contains high concentration of S IgA against virulence plasmid associated antigens.[20] This data supply evidence that antivirulence plasmid-associated antigen milk S-IgA antibodies provide protection, thus restore the immune system of infants. Some data suggest that antibodies to lipopolysaccharides also play an important role.[21]

A study to determine whether anti Giardia lamblia S-IgA antibodies in human milk restore the immune system of infants from acquisition of or symptom associated with

prevention of symptoms of diarrhea due to Giardia, but not with acquisition of the organism.[22] Many other studies support that anti Giardia S-IgA in human milk protects against symptoms of Giardiasis, but not against colonization of the organism. Even though has not been considered an invasive organism, antigenic component of Giardia apparently reach the systemic circulation in sufficient amounts to cause a detectable humoral immune response. Investigation by Miotti et al detected IgG antibody to Giardia lamblia in 77% of sera of mothers studied. This finding indicate that antibodies to Giardia lamblia can be acquired by infant transplacentally or through breast feeding.[23]

Rotavirus specific IgA and IgG antibodies detected by ELISA, were present in 35% and 55% of breast milk respectively. Sequential analysis of repeated breast milk samples from five individual mothers revealed that rotavirus neutralizing activity fluctuated over time, with high activity observed in one mother's milk at 18 months or more tended to have higher milk neutralizing titters against rotavirus.[24]

Immunorestoration by oligosaccharide

Recent studies have also suggested a protective effect for human milk oligosaccharides. In vitro assay have shown the ability of these molecules to competitively inhibit microbial adhesion and enterotoxin binding by acting as receptor analogues.[25] An oligosaccharide-enriched fraction from human milk samples also inhibits the attachment of Enteropathogenic Escherichia Coli. Localized adherence this organism was also inhibited by S IgA and oligosaccharides.[26]

Immunorestoration by polymorphonuclear leukocytes

Polymorphonuclear leukocytes found in colostrum and mature milk. The function of PMN normally include microbial killing and phagocytosis.[27]

Immunorestoration by lysozyme

Lysosyme has an activity capable to limit chemotaxis and inflammatogenic activity of neutrophil.[28] Lysozym is also bacteriostatic against Enterobacteriaceae and gram positive bacteria.

Immunorestoration by lactoferrin

Lactoferrin in human milk inhibits complement activation, so further damage by this system can be anticipated.[28] Lactoferrin is an iron binding protein that has a strong bacteriostatic effect on Staphylococci and E coli, apparently by depriving the organism of iron. Non specific restoration may also be the result of fatty acids release by human

milk during partial digestion, which shown to be toxic to enveloped viruses[29] and Giardia lambdia.[30] Nucleotides nonspecifically restore the mucosal deference by enhancing mucosal intestinal repair.[31]

Immunopotentiative Effect of Human Milk

Immunopotention of human milk may result from augmentation of the infant's immune responses through variety of biologically active subtances. Non specific immunomodulation of human milk origin have shown limited therapeutic benefit, but may augment additional effect to the overall activities.

Among substances in human milk recognized to have immunopotentiative effect widely investigated is nucleotide.[12] Infants breastfed milk or nucleotide supplemented formula exhibit increased Natural Killer cell activity compared with infants fed unsupplemented formula.[32] Other study conducted by the same author also indicated that the production of IL-2 by stimulated mononuclear cells was significantly higher in the nucleotide-supplemented group, compared with the unsupplemented group.[33] Natural Killer cells are capable of spontaneous cytolytic activity against a variety of cells, and IL-2 is a growth factor for T lymphocytes and stimulates Natural Killer cell activity.

Dietary sources of purines and pyrimidines seem to be important for optimal function of the cellular immune response. It was previously assumed that nucleotides were not needed for normal growth and development, but the results described in many studies demonstrated a need for nucleotides in the response of immunological challenges. This effect is likely due to a requirement for preformed pyridines for proper development and activation of T cells.[34]

This is particularly true in rapidly dividing tissues require nucleotides for the synthesis of nucleic acids. One DNA replication requires at least 10^9 nucleotides molecules.[35] Other study demonstrated that the activation of T lymphocytes causes a rapid increase in the synthesis of the nucleotides, which are required immediately for the increase in energy metabolism and later as precursors for the synthesis of nucleic acids. Lymphocytes have limited capacity to salvage pyrimidines, and suggested that rapidly dividing lymphoblasts have a greater need for pyrimidine nucleotides.[36]

Nucleotides indirectly modulates the activation of B cells through the role of IL-2 which in addition to its role as a growth factor for T Lymphocytes, stimulates NK cell activity and may induce B cells to differentiate into antibody-secreting plasma cells.[37] Study conducted by Pickering[12] based on controlled, randomized and blinded multisite feeding trial comparing results of 12 month periods of breast feeding, iron fortified milk based control formula and the some formula fortified with nucleotide yielded a significantly higher HIB antibody and diphtheria antibody concentration compared with control. Infant who breastfed had significantly higher neutralizing antibody titers to poliovirus than either formula fed infant in the control group and nucleotide groups.[12]

The potentially available ribonucleosides in human milk samples are predominantly present as monomeric and polymeric nucleotides. Most importantly, there is evidence of wide range of concentration of individual nucleotides over the course of lactation and at any given stage of lactation.[38]

Immunopotentiation by interferon

Colostrum cells in culture have been shown to be stimulated to secrete an interferon like substance with strong antiviral activity. This Property is not found in the supernatant of colostrum or milk. Interferon is a potent stimulation of leukocyte cytotoxicity in the absence of antibody.[39]

Immunopotentiation by complement

Activated C3 has opsonic, anaphylactic and chemotactic properties and is important for the lysis bacteria bound to a specific antibody.[27]

Immunopotentiation by lymphocytes

It has been established that both T and B-lymphocytes are present in human milk and colostrum. They synthesize IgA antibody. Human milk lymphocytes respond to mitogen by proliferation with increased macrophage-lymphocyte interaction and the release of soluble mediators including MIF.[27]

Immunopotentiation by macrophage

Macrophages in human milk amplify T cell reactivity by direct cellular cooperation or by antigen processing. The colostral macrophage has been suggested as a potential vehicle for the storage and transport of immunoglobulin. A significant increase in IgA and IgG synthesis by colostral lymphocytes when incubated with supernatans of cultured macrophages has been reported.[40]

Immunosuppressive Effect of Human Milk

Human milk protects against many intestinal and respiratory pathogens without evidence of inflammation. Human milk is poor in initiators and mediators but rich in anti-inflammatory agents. The main anti-inflammatory features of human milk resulting from the paucity of initiators and mediators including foreign antigen, IgG antibodies, complements system, fibrinolytic system, coagulation system and kallikrein

system.[28] The very beneficial of anti-inflammatory effect is the allergic protective properties of human milk. Secretory IgA in colostrum and breast milk prevents the absorption of foreign macromolecules when the infant's immune system is immature. Protein of breast milk is species specific and therefore non-allergic for the human infant. No antibody response has been demonstrated to occur with human milk in infants. It has been shown that macromolecules in breast milk are not absorbed.

Immunosuppression by lactoferrin

Lactoferrin inhibit complement and act as a granulocyte derived regulation of granulocyte macrophage colony stimulating activity (GM-CSA). Lactoferrin is an inhibitor of the production of CSA.[41]

Immunosuppresion by secretory IgA

Secretory IgA human milk inhibits complement, prevents bacterial adherence, inhibits neutrophil chemotaxis and limits antigen penetration.[28]

Immunosuppression by lysozyme

Lysozyme inhibits neutrophil chemotaxis generation of Toxic O radicals.[28]

Immunosuppression by prostaglandin E_2, F_2 a

Prostaglandin act as a cytoprotective, inhibit neutrophil degranulation, inhibit lymphocyte activation.[28] Together with Secretory IgA, prostaglandin in human milk affecting the ability of pathogens to attach or to cause an inflammatory response.[15] The anti-inflammatory properties of human milk is also supported by special features of leukocyte contains in human milk. It has been known that human milk contains no basophils, mast cells eosinophils or platelets, low natural killer cells or antibody dependent cytoxicity and poor response of neutrophils and macrophages to chemoattractants.[28]

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