

# Breast Milk Immunoprotection and the Common Mucosal Immune System: a Review

Prameela KK<sup>1</sup> & Mohamed AEK<sup>2</sup>

<sup>1</sup> *Consultant Pediatrician, Clinical Department*

<sup>2</sup> *Professor of Immunology and Parasitology, Preclinical Department II  
Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, 55100 Kuala Lumpur,  
Malaysia*

## ABSTRACT

It is universally known that breastfeeding provides a broad spectrum of nutritional and non-nutritional advantages to the developing infant. Non-nutritive protecting potentials of breast milk towards different infections and non-infectious diseases are still attracting the attention of researchers in different specialties. The neonate, who is suddenly exposed to a wide variety of organisms, is in dire need of protection, enhancement and education of his immature immune system to encounter these organisms. The lactating mammary gland is an integral part of the common mucosal immune system which stands as a sentinel in combating pathogens that enter the body via the mucosal route. The common mucosal immune system also competently controls tolerance mechanisms to innocent proteins and is involved in surveillance of carcinogenesis. The diverse roles of general mucosal immunity are nearly well established but the specialised functions of breast tissue and breast milk in boosting the immune responses need more emphasis and highlighting. The detailed understanding and evaluation of breast milk as an immunological tool is reviewed within the domain of the diverse activities of the common mucosal immune system.

**Keywords:** Breastfeeding, breastmilk, common mucosal immune system, immunity, lactating mammary gland

## INTRODUCTION

Breastfeeding is globally recognised to be an exclusive nutrient for the first six months of the infant's life and as a supportive nutrient for a further eighteen months more. This is based on the fact that the immune and nutritive factors in breast milk confer immediate and long term benefits which are further evidenced by the observation that those who had been deprived of breastfeeding in infancy suffer many morbid

conditions in their adult life (Drash *et al.* 1994). In spite of abundant information about the constituents of breast milk, many facets remain unclear and the precise role of lactating mammary glands in mucosal immunity is actually understated and perhaps, understudied.

## MUCOSAL PROTECTION

Mucosal surfaces or mucosae are organised epithelial structures found throughout the

body and formed by sheets of cells which are continually bathed in a thick layer of mucus. They line the gastrointestinal, respiratory and urogenital tracts and are found in the pancreas, conjunctivae, lachrymal glands, salivary glands, mammary glands and body orifices. Mucosae cover, protect and provide secretory and absorptive functions through moist mucoid surfaces. The nature of cells forming a particular mucous membrane reflects the specialised functions of that tissue; however all mucosae share a common function of interaction between the internal and external environments of the body. Moreover, mucosae form the largest surface area of the body and a very important portal of entry for pathogens during inspiration, upon ingestion, or when in contact with the conjunctiva or the genitourinary mucosal surfaces. Protection at the mucosal surfaces is a very intricate process which depends on a complex barrier of non-cellular components and the integral structure of the common mucosal immune system (Abraham, Sharon & Ofek, 1999; Nagler-Anderson, 2001).

Non-cellular mucosal barriers are different interrelated components with mucin representing the key structure. Mucin is a mixture of glycoproteins, proteoglycans, peptides and enzymes (Lamont, 1992). The glycoproteins of mucin line the surface epithelium from the oro-nasal cavity to the rectum forming a thick barrier that traps the invading bacteria and viruses to be expelled later in a process called 'non-immune exclusion' (Belley *et al.*, 1999).

Another significant non-cellular mucosal barrier is formed by gut enzymes found throughout the length of the tract. A set of active enzymes are produced by simple and complex gland structures under tight control of tactile stimulation of mucosa, local intrinsic nervous plexus reflexes, parasympathetic stimulation and group of autocrine hormones (McNabb & Tomasi, 1981). Moreover, extraordinarily

stable small proteins known as 'trefoil factors' help to strengthen the barrier function of mucosa and promote its restoration if any defect occurs (Wright, 1993).

The common mucosal immune system is perfectly designed as an integrated network of lymphoid tissues, mucous membrane associated cells and a collection of effective molecules, existing alongside but separate from the peripheral immune system in the blood. It presents well-tuned defense elements; the first is compact and localised while the second is more diffuse and disperse (Brandtzaeg, Baekkerold & Farsted, 1999). The compact component is concerned with selective picking of foreign antigens and introducing them into exclusive antigen processing cells to initiate the targeted responses while the second diffuse component is a collection of effector B- and T lymphocytes, differentiated plasma cells, macrophages, dendritic cells, eosinophils, basophils and mast cells which work together to accomplish more organised host protection (Conley & Delacroix, 1987). Finally, the components of the mucosal immune system interact together to produce effective cell-mediated and humoral protective responses, or under certain conditions, produce systemic anergy known as 'mucosally introduced tolerance' (Weiner, 1997).

### **THE MUCOSA-ASSOCIATED LYMPHOID TISSUE (MALT)**

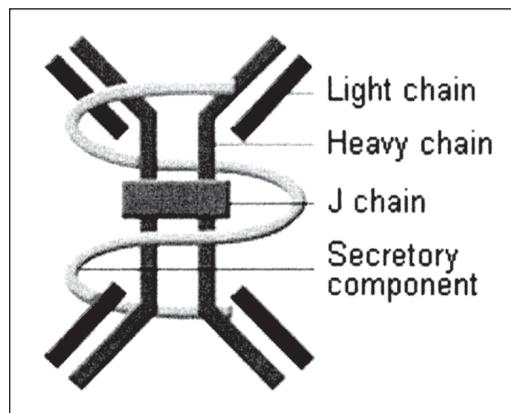
The mucosal surfaces in the adult are extensive, and require significant supportive expenditures of lymphoid tissues and other effector molecules. These structures form the core ingredient of the common mucosal immune system and are collectively known as mucosa-associated lymphoid tissue MALT (Langkamp-Henken, Glezer & Kudsk, 1992). MALT is not a single structure; there are different divisions incorporating diverse locations which carry a key to their

functions. Thus, MALT comprises gut-associated lymphoid tissue (GALT), bronchial/tracheal-associated lymphoid tissue (BALT), nasal-associated lymphoid tissue (NALT), vulvovaginal-associated lymphoid tissue (VALT), larynx associated lymphoid tissue (LALT), skin-associated lymphoid tissue (SALT), conjunctiva-associated lymphoid tissue (CALT), mammary glands associated lymphoid tissue and the products of lactation (Kracke *et al.*,1997; Kelsall & Strober, 1999). The organised lymphoid follicles in the GALT and BALT are considered the principal inductive sites of mucosal immune response in the body (Mc Ghee, Lamm & Strober,1999). Structurally, MALT is formed of intra-epithelial CD8+ lymphocyte repertoire, B-cells, dendritic cells, macrophages and microfold cells, (M-cells). M cells are of special interest as they are found in the mucosal inductive sites in the intestinal and upper respiratory tract and stand as sentinels to mucosal structures. They lack microvilli and glycocalyx coating and exhibit microfolds on their luminal surface; these characters suite their functions to absorb, transport, process and present antigens to subepithelial lymphoid cells (Featherstone,1997). Interestingly, the pathogenic reovirus, invasive salmonella and shigella

can gain entry via M-cells into deeper MALT structures; some studies focus on identification of the exact bacterial and viral virulence factors associated with targeting M cells, like sigma protein of reovirus, aiming to develop effective vaccines and vaccine components that could be delivered directly into mucosal inductive sites (Wolf *et al.*,1981). In the evolutionary process, different MALT structures are developed at birth, with discrete T and B-cell populations apparent as early as 19 weeks gestation. However secondary follicles with germinal centers reflecting B-cell activation do not appear until weeks after birth, reflecting their dependency on exogenous stimuli (Brandtzaeg, 1998; Brandtzaeg,2003).

### IMMUNOGLOBULIN A (IgA)

The remarkable magnitude of GALT as an inductive site for mucosal humoral immunity is due to the fact that about 80% of all IgA-producing blasts and plasma cells are located in the intestinal lamina propria, with a daily production of 3-5g of IgA flowing into the intestinal lumen (Cebra *et al.*,1977). IgA is the major humoral component of mucosal immunity found mainly associated with mucosal surfaces and expressed after B cells undergo class-



**Figure 1.** The molecular structure of sIgA (adapted from: Robyn Seipp, 2003)

switching driven by cytokine stimulation (Van Cott *et al.*,1996). The secreted IgA is a complex polypeptide dimer of two basic heavy chains held to two light chains by J chain. When IgA is transported across the mucosal epithelium, it will acquire an additional polypeptide component and polymeric IgA (pIgA) is then formed. pIgA is transported by polymeric Ig receptor (pIgR) into mucosal secretions and extracellular domain of pIgR will be bound to it to protect the molecule from proteolytic enzymes in the harsh gastrointestinal tract environment (Kaetzel *et al.*,1991). In the end, secretory IgA (sIgA) synthesis comes as a net result of complicated molecular reactions between epithelial cells, IgA-committed B cells and Th cells (McIntyre & Strober, 1999).

As sIgA is the main effector humoral component of the mucosal immune system, its molecular structure offers significant stability which fits its professional function of mucosal protection (Woof & Mestecky, 2005). In the mucosal lining, sIgA mediates a variety of 'immune exclusion' functions by interfering with bacteria and virus adherence under tactical process known as 'intracellular neutralisation' (Yan *et al.*, 2002).

Because sIgA functions as an inhibitor of bacterial and viral attachment to the mucosal epithelium and agglutinates antigens, it is viewed as a benign antibody which fails to bind complement and in turn, unable to elicit an inflammatory response (Macpherson *et al.*,2001). sIgA is released into serum in small amounts as a monomer molecule which may function as a second line of defense by eliminating pathogens that have breached the mucosal surface (Woof & Kerr, 2007).

The sIgA typically interacts with Fc receptor (Fc $\alpha$ R1) on the surface of the invading pathogens triggering antibody-dependent-cell-mediated cytotoxicity (ADCC) which offers additive protection against many bacteria and viruses including HIV-1 (Mantiset *et al.*,2007). Thus, it can be

concluded that the hallmark of the mucosal immune response is the development of sIgA with vital participation of other immunoglobulin isotypes and cellular elements, including IgE, intraepithelial lymphocytes, dendritic cells, T-lymphocytes subsets, and a spectrum of immunoregulatory and proinflammatory cytokines and chemokines available locally in the mucosa (Beagley & Elson, 1992).

## BREAST MILK AND sIgA

In humans, the production and functions of sIgA are not optimum until four years of age, however breast milk is able to compensate this when it confers sIgA passively to the suckling infant, providing a robust local immunity. sIgA in the breast milk, mainly colostrum, is a developmental bridge until the infant's intestines secrete its own. The known protective effects of colostrum against diarrheal diseases led some references to define neonatal diarrhea as a 'colostrum deficiency syndrome'. This encouraged some researchers to try therapeutic doses of colostrum (5 mg/kg daily) and found it able to reduce neonatal *Escherechia coli* and other viral diarrhea (Jelliffe & Jelliffe,1981). The profile of sIgA production in breast milk is found to be influenced by maternal age, mood variables, immunological and infectious factors, serum proinflammatory and proimmune cytokines and cortisol levels. Older and stressed women showed lower sIgA while positive life events were correlated with higher breast milk sIgA levels (Groer, Davis & Steele,2004). An important compensatory mechanism in IgA deficient infants appears to be offered by IgM (Mellander *et al.*,1986). Investigations of human mucosa have shown that clonally related IgM+ plasma cells probably secrete antibodies with similar specificities in the same mucosal micromilieu (Mbawuikie *et al.*,1999). Mucosal IgM-IgA interactions have been studied in murine models and have proven the presence of active switching

from IgM to IgA in the gut mucosa micro-environment (Chapman *et al.*,1996 and Fagarasan *et al.*,2001).

### **THE LACTATING MAMMARY GLAND AS A PART OF THE COMMON MUCOSAL IMMUNE SYSTEM**

The lymphoid tissues found all over the body are classified into primary or central tissues found in hematopoietic bone marrow, and secondary or peripheral tissues located in most strategic points of the body namely the lymph nodes, spleen, MALT, GALT, BALT, NALT, SALT, VALT, urinary tract-associated lymphoid tissue, CALT and lactating mammary glands (Kunisawa, Fukuyama & Kiyono,2005; Randall, Carragher & Rangel-Moreno, 2008). The secondary lymphoid tissues have specialised architecture and micro-environments that promote the controlled interactions of immunocytes to elicit brisk appropriate immune responses (Kunisawa & Kiyono,2005). Secondary lymphoid tissues differ functionally, so while lymph nodes collect antigens and antigen presenting cells from non-lymphoid organs through afferent lymphatics, other mucosal associated lymphoid tissues acquire antigens directly across the mucosa. Secondary lymphoid tissues develop during embryogenesis or in the first few weeks after birth according to highly coordinated interactions between new emerging hematopoietic cells and immature mesenchymal or stromal cells. These interactions are orchestrated by chemokines, cytokines and growth factors that attract hematopoietic cells to the sites of future lymphoid structures and promote their survival and differentiation there (Baggiolini, Dewald & Moser,1997). The lactating mammary glands are integral parts of the common mucosal immune system as evidenced by the milk composition of lymphocytes derived and migrate from precursor immuno-competent cells present in breast-associated lymphoid

tissue and gut-associated lymphoid tissue. The local lymphocytes in the lactating breast tissues are mostly CD4 and CD8 T-cells with less numbers of B lymphocytes. Chemokines and cytokines of transforming growth factor-beta (TGF-beta), interleukin-4 (IL-4) and intrleukin-10 (IL-10) promote B-cell switching into IgA producing cells locally in the lactating mammary glands (Hayward,1983).

Breast milk is a complex mixture of interacting compounds that differ in composition not only between women but also within the lactation period. It is a species specific product which contains proteins, non-protein nitrogen compounds, carbo-hydrates, lipids, minerals and vitamins. Many of the milk components are non-nutritive elements, designed particularly to support immune-development, tolerance, and to regulate inflammation (Oddy, 2001).

### **NON-NUTRITIVE PROTECTIVE VALUES OF BREAST MILK**

Milk is a communication vehicle between the maternal immune system and infant. Although infants have antibodies derived from the maternal circulation, they remain unprotected when challenged with new organisms. Breast milk can minimise this risk by supplying antibodies and by directing and educating the immune, metabolic, and microflora systems of the infant (Brandtzaeg, 2003). The profile of passive immunity transmitted by breast milk to the infant, reflects previous maternal exposures, while the sensitised lymphocytes derived from the breast associated lymphoid tissues show adaptive memory with production of more specific immunoglobulins and cytokines (Bottcher *et al.*, 2000). So, in nursing mothers, plasma cells migrate from activated B-cells to home in the lactating mammary glands, synthesise immuno-globulins, mainly sIgA, and to less extent IgD, IgM, IgE and IgE to provide the suckling infant with an

exceptionally active mucosal protective factors (Field, 2005).

More bioactive factors are also found in the breast milk including lactoferrin, lysozymes, lactoperoxidase, free fatty acids, complement, vitamin B12 binding protein, folic acid-binding protein, bifidus factor, fibronectin, oligosaccharides, high molecular weight mucins and interferons. They have no intrinsic memory but contribute in the non specific protective values of breast milk (Newman, 1995).

Cellular defensive values of breast milk depend on the phase and stage of lactation; varieties of leukocytes appear in colostrum and mature milk, with macrophages forming 55-60% of total breast milk cells, neutrophils 30-40% and lymphocytes 5-10% (Goldman, 1993). Viable breast milk leukocytes have been isolated from the faeces of suckling infants with their key surface molecules remaining antigenically intact. This could be of particular significance in relation to mucosal protective values of breast milk (Goldman, 1993).

Macrophages derived from breast milk modulate the infant's T and B cells through their activation markers (CD11c) which provide phagocytic and immunoregulatory functions. Moreover, these cells contain engulfed sIgA which could be released once in contact with bacteria in the gut (Rivas, El Mohandes & Katona, 1994).

Neutrophils of breast milk demonstrate decreased adherence, polarity, motility and express high levels of CD11b and low levels of L-selectin markers. These criteria are indicative of prior activation and limited functional capacity once they are secreted into milk (Kim *et al.*, 2003).

Lymphocytes in the breast milk show higher proportion of CD8+ and  $\gamma\delta$ + expressed receptors. CD4+ cells expressing CD40L, sCD30 and CD45RO+ surface proteins are also found in the human milk which signify active immunological memory (Eglinton, Robertson & Cummins, 1994).

Field (2005) found CD4+ cells from maternal origin able to compensate the

immature functions of the defective neonatal T cells and promote their maturation. Cytokines in breast milk include IL-4, IL-10 and TGF-beta, which are produced by mucoepithelial cells and imply collaboration between neighboring lymphocytes and mucosal microenvironment of the breast associated lymphoid tissues (Field, 2005).

Lactoferrin is an iron-binding protein with a hundredfold higher activity than transferrin, the major iron transport protein in the body. The highest known concentration of lactoferrin is 7 gram /liter which is found in human colostrum only. Mature human milk contains about 1 gram/liter and bovine milk contains lesser amounts (Ward, Paz & Conneely, 2006). Lactoferrin has different biological activities, it inhibits different pathogenic bacteria, viruses, parasites and fungi, activates a variety of immune system cells, regulates normal cell growth and inhibits tumor cell division and spreads in experimental animals (Weinberg, 2002). In the breast milk, Lactoferrin helps the infant to absorb more iron, and seems essential factor for B- and T- lymphocytes growth (Valenti & Antonini, 2006).

Alpha-lactalbumin is a protein in breast milk found to destroy cancer cells (Jensen, & Lammi-Keefe, 2001). This may partly explain the early report on childhood lymphoma being nine times higher in bottle-fed infants (Davis, Savitz & Grauford, 1988).

## OTHER PROTECTIVE FACTORS

Prostaglandins in fresh breast milk are believed to protect the immature delicate gastrointestinal mucosa of the newborn. Premature babies are partially protected against necrotising enterocolitis (NEC) when they are breastfed (Bedrick, 1990 and Lucas & Cole, 1990). Lactadherin is a glycoprotein which attaches to rotaviruses and inhibits their settling on the gut wall (Newburg *et al.*, 1998). Interferons, found primarily in colostrum, have strong anti-viral activity (Bocci *et al.*, 1993). Colostrum fibronectins intensify phagocytes to attack bacteria

aggressively even before being tagged by antibodies (Friss *et al.*,1988). Cortisol and other smaller proteins of epidermal growth factor, nerve growth factor, insulin-like growth factor and somatomedin C, act to close up the leaky mucosal lining of the infant, making it relatively impermeable to pathogens (Newman,1995).

### **BREAST MILK PROMOTES TOLERANCE AND PRIMING OF THE INFANT'S IMMUNE SYSTEM**

The mechanisms responsible for sensitisation, in particular within the gastrointestinal tract, are IgE-mediated as well as of a non-IgE-mediated, immunological origin. The phenomenon that is the opposite of sensitisation is the maintenance of tolerance and is exemplified by 'oral tolerance'. The cytokines transforming growth factor beta and interferon gamma have been shown to be key immunoregulatory cytokines in oral tolerance (Kalliomäki *et al.*, 1999). Lack of maturation of the infant immune system from a T helper 2 to a T helper 1 type of immune response may be caused by less microbial challenge especially in formula milk fed infants (Steffen,2001). Apart from the genetic constitution of the individual, breastfeeding in infancy may be the most important single determinant for the development of clinical tolerance. During breastfeeding, there is a fine balance between antigen responses that results in tolerance, and the responses which result in sensitisation. Successful development of tolerance contributes to lower incidences of food-related allergies in breast-fed infants (Van Odijk *et al.*, 2003).

### **CONCLUSION**

The impressive facts about breast milk should consolidate firm efforts towards continuous breast feeding in infants for up to two years. Feeding decisions are very individual, but the current knowledge about the unique composition and indispensable

values of breast milk, makes it undoubtedly the first choice. The mother's mature and more effective immune system is represented successfully in the lactating mammary glands as an integral part of the common mucosal immune system. Breast milk then confers passive and active immunities to the suckling infant. Yet no artificially engineered formula can offer the complete set of breast milk ingredients. Furthermore, human breast milk as a species specific product cannot be equalised to any other animal milk. The biochemical, physiological and genetic backgrounds of breast milk components should be further analysed and more studies on mucosal associated lymphoid tissues must focus on the lactating mammary gland. It is hoped that further research in this area may improve the understanding of the complex strategies of mucosal immunity provided by breast milk which carries a special importance for nutritionists, immunologists and pediatricians alike.

### **REFERENCES**

- Abraham S, Sharon N & Ofek I (1999). Adhesion of bacteria to mucosal surfaces. In: *Mucosal Immunology*. Ogra PL, Mestecky J, Lamm M, Strober W, Bienenstock J, McGhee J (eds)(2<sup>nd</sup> ed). Academic Press, pp. 31-42, New York.
- Baggiolini M, Dewald B & Moser B. (1997). Human chemokines: an update. *Ann Rev Immunol* 15: 675-705.
- Beagley K & Elson C (1992). Cells and cytokines in mucosal immunity and inflammation. *Gastroenterol. Clin. North Am* 21(2) : 347-366.
- Bedrick A (1990). The physiologic significance of milk-borne prostaglandins for the developing gastrointestinal tract. *Mammary Gland Biology and Lactation Newsletter* 9(4).

- Belley A, Keller K, Gottke M, Chadee K & Goettke M (1999). Intestinal mucins in colonization and host defense against pathogens. *Am J Trop Med Hyg* 60 (suppl.4):10–15.
- Bocci V, von Bremen K, Corradeschi F, Franchi F, Luzzi E & Paulesu L (1993). Presence of interferon-gamma and interleukin-6 in colostrum of normal women. *Lymphokine Cytokine Res* 12 (1): 21–24
- Bottcher M, Jenmalm M, Garofalo R & Bjorksten B (2000). Cytokines in breast milk from allergic and nonallergic mothers. *Pediatr Res* 47:157–162.
- Brandtzaeg P (1998). Development and basic mechanisms of human gut immunity. *Nutr Rev* 56: S5-S18.
- Brandtzaeg P (2003). Mucosal immunity: integration between mother and the breast-fed infant. *Vaccine* 21: 3382–3388.
- Brandtzaeg P, Baekkerold E & Farsted I (1999). Regional specialisation in the mucosal immune system: what happens in the microcompartment? *Immunol Today* 20: 141–151.
- Catherine J. Field (2005) The immunological components of human milk and their effect on immune development in infants. *J Nutr* 135:1–4
- Cebra J, Kamat R, Gearhart P, Robertson S & Tseng J (1977) The secretory IgA system of the gut. *Ciba Found Symp* 26-28; (46): 5–28.
- Chapman C, Mockridge C, Hamblin T, & Stevenson F (1996). Tracking of the V4-34 (VH4-21) gene in human tonsil reveals clonal isotype switch events and a highly variable degree of somatic hypermutation. *Clin Exp Immunol* 105 (2): 360–368.
- Conley M & Delacroix D (1987). Intravascular and mucosal immunoglobulin A: two separate but related systems of immune defense. *Ann Intern Med* 106: 892–899
- Davis M, Savitz D & Grauford B (1988). Infant feeding and childhood cancer. *The Lancet* 2(8607): 365–368.
- Drash A, Kramer, M, Swanson J & Udall, J (1994). Infantfeeding practices and their possible relationship to the etiology of diabetes mellitus. *American Academy of Pediatrics* 94 (5): 752–754.
- Eglinton B, Robertson, D & Cummins, A (1994). Phenotype of T cells, their soluble receptor levels, and cytokine profile of human breast milk. *Immunol Cell Biol* 72: 306–313.
- Fagarasan S, Kinoshita K, Muramatsu M, Ikuta K & Honjo T (2001). *In situ* class switching and differentiation to IgA-producing cells in the gut lamina propria. *Nature* 413:639–643
- Featherstone C (1997). Cells: portals to the mucosal immune system. *Lancet* 25; 350 (9086): 1230.
- Friss H, Rubin L, Carsons S, Baranowski J & Lipsitz P (1988). Plasma fibronectin concentrations in breast fed and formula fed neonates. *Arch Dis Child* 63(5): 528–532.
- Goldman AS (1993). The immune system of human milk: antimicrobial, anti-inflammatory and immunomodulating properties. *Pediatr Infect Dis J* 12: 664–671.
- Groer M, Davis M & Steele K (2004). Associations between human milk SIgA and maternal immune, infectious, endocrine, and stress variables. *J Human Lactation* 20(2): 153–158.

- Hayward, AR (1983). The immunology of breast Milk. In : *Lactation: Physiology, Nutrition and Breast-Feeding*. Nville, M & Neifert, M(eds). pp. 249–272. Plenum Press, New York.
- Jelliffe DB & Jelliffe EF (1981). Breast milk and infection. *Lancet* 2 (8243): 419.
- Jensen RG & Lammi-Keefe C (2001). The anticarcinogenic conjugated fatty acid c9, t11–c18:2, or ruminic acid, in human milk: amounts and effects. *Adv Exp Med Biol* 501: 153–156.
- Kaetzel C, Robinson J, Chintalacharuvu K, Vaerman J & Lamm M (1991). The polymeric immunoglobulin receptor (secretory component) mediates transport of immune complexes across epithelial cells: a local defense function for IgA. *Proc Natl Acad Sci USA* 88 (19): 8796–8800.
- Kalliomäki M, Ouwehand A, Arvilommi H, Kero P & Isolauri E (1999). Transforming growth factor- $\beta$  in breast milk: A potential regulator of atopic disease at an early age. *J Allergy Clin Immunol* 104: 1251–1257.
- Kelsall B & Strober W (1999). Gut-associated lymphoid tissue: antigen handling and T-lymphocyte responses. In: *Mucosal Immunology*. Ogra P, Mestecky J, Lamm M, Strober W, Bienenstock J, McGhee J (eds)(2<sup>nd</sup> ed.) Academic Press, pp. 293–317. New York.
- Kim S, Keeney S, Alpard S & Schmalstieg F (2003). Comparison of L-selectin and CD11b on neutrophils of adults and neonates during the first month of life. *Pediatr Res* 53:132–136.
- Kracke A, Hiller A, Tschernig T, Kasper M, Kleemann W, Tröger H & Pabst R (1997). Larynx-associated lymphoid tissue (LALT) in young children. *Anat Rec* 248: 413–420.
- Kunisawa J, Fukuyama S & Kiyono H (2005). Mucosa-associated lymphoid tissues in the aerodigestive tract: their shared and divergent traits and their importance to the orchestration of the mucosal immune system. *Curr Mol Med* 5: 557–72
- Lamont J (1992). Mucus: the front line of intestinal mucosal defense. *Ann NY Acad Sci* 664:190–201.
- Langkamp-Henken B, Glezer J & Kudsk K (1992). Immunologic structure and function of the gastrointestinal tract. *Nutr Clin Pract* 7 (3): 100–8.
- Lucas A & Cole T (1990). Breast milk and neonatal necrotising enterocolitis. *The Lancet* 336 (8730): 1519–23.
- Macpherson A, Hunziker L, McCoy K & Lamarre A (2001). IgA responses in the intestinal mucosa against pathogenic and non-pathogenic microorganisms. *Microbes Infect* 3:1021–1035
- Mantis N, Palaia J, Hessel A, Mehta S, Zhu Z, Corthésy B, Neutra M, Burton D & Janoff E (2007). Inhibition of HIV-1 infectivity and epithelial cell transfer by human monoclonal IgG and IgA antibodies carrying the b12 V region. *J Immunol* 179: 3144–3152.
- Mbawuiké I, Pacheco S, Acuna C, Swtzer K, Zhang Y & Harriman J (1999). Mucosal immunity to influenza without IgA: an IgA knockout mouse model. *J Immunol* 162: 2530–7.
- McGhee J, Lamm M & Strober W (1999). Mucosal immune responses: an overview. In: *Mucosal Immunology*. Ogra P, Mestecky J, Lamm M, Strober W, Bienenstock J, McGhee J (eds)( 2<sup>nd</sup> ed). Academic Press, pp. 485–506. New York.

- McIntyre T & Strober W (1999). Gut-associated lymphoid tissue: regulation of IgA B-cell development. In: *Mucosal Immunology*. Ogra P, Mestecky J, Lamm M, Strober W, Bienenstock J, McGhee J (eds) (2<sup>nd</sup> ed). Academic Press, pp. 507–510. New York.
- McNabb P & Tomasi T (1981). Host defense mechanisms at mucosal surfaces. *Annu Rev Microbiol* 35: 477–496.
- Mellander L, Björkander J, Carlsson B & Hanson LA (1986). Secretory antibodies in IgA-deficient and immunosuppressed individuals. *J Clin Immunol* 6 (4): 284–91.
- Nagler-Anderson C (2001). Man the barrier! Strategic defences in the intestinal mucosa. *Nat Rev Immunol* 1: 59–67.
- Newburg D, Peterson J, Ruiz Palacios G, Matson D, Morrow A & Shults J (1998). Role of human-milk lactadherin in protection against symptomatic rotavirus infection. *The Lancet* 351(9110): 1160–1164.
- Newman J (1995). How breast milk protects Newborns. *Scientific American* 273 (6): 76.
- Oddy W (2001). Breastfeeding protects against illness and infection in infants and children: a review of evidence. *Breastfeed Rev* 9 (2): 11–8.
- Rivas, R, El Mohandes, A & Katona I (1994). Mononuclear phagocytic cells in human milk: HLA-DR and Fc gamma R ligand expression. *Biol Neonate* 66: 195–204.
- Steffen H (2001). Sensitization and tolerance. *Current Opinion in Allergy and Clinical Immunology* 1 (3): 237–241.
- Troy D Randall, Damian M Carragher & Javier Rangel-Moreno (2008). Development of secondary lymphoid organs. *Annu Rev Immunol* 26: 627–650.
- Valenti P & Antonini G (2006). Lactoferrin: an important host defence against microbial and viral attack. *Cell Mol Life Sci* 62 (22): 2576–87.
- Van Cott J, Staat H, Pascual D, Roberts M, Chatfield S & Yamamoto M (1996). Regulation of mucosal and systemic antibody responses by T helper subsets, macrophages, and derived cytokines following oral immunization with live recombinant salmonella. *J Immunol* 156: 1504–14.
- Van Odijk J, Kull I, Borres M, Brandtzaeg P, Edberg U, Hanson L, Host A, Kuitunen M, Olsen S, Skerfving S, Sundell, J & Wille S (2003). Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966-2001) on the mode of early feeding in infancy and its impact on later atopic manifestations. *Allergy* 58: 833–843.
- Ward P, Paz E & Conneely O (2006). Multifunctional roles of lactoferrin: a critical overview. *Cell Mol Life Sci* 62 (22): 2540–8.
- Weiner H (1997). Oral tolerance: immune mechanisms and treatment of autoimmune diseases. *Immunol Today* 18:335–343.
- Weinberg E (2002). Human lactoferrin: a novel therapeutic with broad spectrum potential. *J Pharm Pharmacol* 53 (10): 1303–10.
- Wolf J, Rubin D, Finberg R, Kauffman R, Sharpe A, Trier J & Fields B (1981). Intestinal M cell: a pathway for entry of reovirus into the host. *Science* 212: 471–2.

- Woof J & Mestecky J (2005). Mucosal immunoglobulins. *Immunol Rev* 64–82.
- Woof J & Kerr M (2007). The function of immunoglobulin A in immunity. *J Pathol* 208 (2): 270–82. Review.
- Wright N (1993). Trefoil peptides and the gut. *Gut* 34(5):577–9.
- Yan H, Lamm M, Bjorling E & Huang Y (2002). Multiple functions of immunoglobulin A in mucosal defense against viruses: an in vitro measles virus model. *J Virol* 76:109–72.