Review

A viewpoint of mucosal immunity in relation to early feeding method

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Summary Atopic diseases are common health problems in society and their incidence is increasing unabated. A number of studies in animal models have shown that antigen-specific IgE suppression could be induced for the treatment of allergic disorders. Many of the present therapeutic strategies in children have not been entirely successful and early breastfeeding could provide a practicable means of helping the affected children. An overview of the mucosal immune system is hereby presented to explain the natural mechanisms involved in protecting organisms from allergic reactions to food and other non-harmful antigens presented at the mucosal surfaces. The protective role of early breastfeeding in modulating this natural phenomenon is emphasized. The undeniable limitations of breastfeeding in the management of some peculiar cases of childhood dietary protein intolerance are also highlighted.

Keywords Allergy, atopy, breastfeeding, mucosal immunity, neonatal immunity, oral tolerance.

Introduction

One of the earliest clinically important problems seen in infants that are at high risk for developing atopy is the development of signs of cow-milk proteins intolerance. Twenty to thirty percent of the population are known to be suffering from one form of allergy or another, and the incidence is continually increasing, especially in the developed countries (Holt, 1996; Strannegard & Strannegard, 1999).

Efforts geared towards the management of these allergies have so far been concentrated mainly on allergen avoidance and production of hypoallergenic milk formulae for the feeding of affected infants and those with high risks for developing atopy. Many of the protein-hydrolysate formulae, however, still contain significant amounts of the allergenic milk proteins, such as β-lactoglobulin (Chiancone *et al*., 1995). Also, a high percentage of allergic infants fail to positively respond to any of the extensively hydrolysed protein formulae (Businco *et al*., 1989, 1994; Vaarala *et al*., 1995). Further disadvantages of these artificial formulae include the risks of growth or metabolic disturbances to the nursing infant, as well as their high prices (Bjorksten, 1984; Giovannini *et al*., 1994).

Recent advances in protein technology have made it possible for the development of infant formulae based on elemental L-amino acids. These formulae are better tolerated and are effective in the management of children with severe symptoms and cases of multiple food protein intolerance (Isolauri *et al*., 1995; Vanderhoof *et al*., 1997; Hill *et al*., 1999a). Incidentally, these amino-acid based formulae have also been found to be effective in alleviating unremitting symptoms of gastroesophageal reflux and eosinophilic oesophagitis (Kelly *et al*., 1995; Heine *et al*., 2000). Infant formulae based on chicken or lamb-meat protein have also

Abbreviations LP – lamina propria, mLN – mesenteric lymph nodes, PP – Peyer's patches, GALT – gut associated lymphoid tissues, MALT – mucosal associated lymphoid tissues, OT – oral tolerance.

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been found to be less allergenic and useful in the management of children who are allergic to the hydrolysate formulae (Weisselberg *et al*., 1996).

Recommendation of prolonged exclusive breastfeeding of high-risk infants on the other hand, has been shown to provide both short- and long-term benefits in reducing the incidence of atopic disorders in infancy and later in childhood. Only few studies, mainly from the developed countries, have reported cases in which breastfeeding have actually aggravated symptoms of childhood protein allergies (Isolauri *et al*., 1999). The differences in the pattern and prevalence of atopic diseases observed between the poor developing and industrialized countries might be related to both genetic and cultural factors such as level of consumption of polyunsaturated fatty acids (Hill *et al*., 1999b; Kankaanpaa *et al*., 1999).

Generally, the exact mechanisms underlying the development of food-related allergies on the one hand, and the immunomodulatory role of early breastfeeding on the other hand, are only incompletely understood. However, the risk of manifest intestinal atopy is generally increased by early intake of foreign allergens in the presence of genetic predisposition (Sasai *et al*., 1994). It is now evident that the immune system of both atopic and normal individuals are capable of responding to various nonpathogenic allergens right from late foetal and early postnatal periods, with a predominant pattern of Th2 antibody production, including IgE (Sasai *et al*., 1994; Holt, 1996).

Early breastfeeding provide both passive and active protection to the immature immune system of the infant by the avoidance of early introduction of allergenic substances and provision of adult-type immune deviation mechanisms, respectively. Several mechanisms can be identified which account for the protection offered by breastfeeding against infections and immunological diseases, in addition to several other economical, nutritional, psychosocial and cognitive developmental advantages.

Anatomic structure of mucosal immunity

The lymphoid tissue in the mucosa occur as loosely constituted lymphocytes isolated throughout the interstitium, particularly concentrated in the lamina propria (LP) (Bienenstock *et al*., 1983).

Intra-epithelial lymphocytes consist almost exclusively of T lymphocytes in the epithelium of the intestine and other mucosal surfaces. They primarily express suppressor/cytotoxicity (CD8) phenotypes, capable of antibody-dependent cytotoxicity, T cell-cytotoxicity and natural killer (NK) cell functions. Fifty percent of the cells also contain granules of sulphated mucopolysaccharides and sometimes histamine (Goodacre *et al*., 1979). The exact nature of their function and mechanisms of action is however not known with any certainty. Their close association with intestinal epithelial cells, which are known to express mononuclear cells (MHC) class 2 (HLA-D/DR) antigens, might suggest that these cells are capable of presenting antigens to the T-lymphocytes, thereby eliciting an immune response against invading foreign antigens.

The mucosa also contains solitary sub-epithelial lymphoid nodules scattered throughout the length of the intestine and much of the upper and lower respiratory tracts. In addition, localized aggregates of lymphoid follicles known as gut-associated lymphoid tissue (GALT), are also present in the lower part of the small intestinal Peyer's patches (PP), tonsil and appendix. A similar structure in the respiratory tract is the bronchus-associated lymphoid tissue (BALT). These lymphoid nodules contain predominantly B cells which are immunoglobulin A (IgA) surface immunoglobulin positive, and few thymus-dependent areas, particularly T-helper cells preferentially specific either for IgA alone or IgA and IgM with a smaller proportion of suppressor/cytotoxic subsets.

The epithelium overlying these lymphoid nodules contain specialized microfold M-cells which are highly specialized epithelial cells that pinocytose antigens and present them to the underlying lymphoid tissues (Bockman *et al*., 1983). The GALT represent a site both for primary immune response to antigens and for the amplification of IgA production by cells primed elsewhere. The PP lymphoid aggregates seems to be the primary site of B cell priming, which then migrate to the LP of the intestinal and other sub-epithelial sites. The IgA-producing plasma cells in the submucosa account for 49–84% of cells identified by immunohistochemical staining for intracytoplasmic immunoglobulins. The proportion of $IgA2^{+}$-plasma cells in the

distal gut, mammary gland and salivary glands ranges between 34 and 59%, while they constitute only 5–9% in the peripheral lymph nodes (PLN), spleen and tonsil.

Mucosa associated lumphoid tissues (MALT) antigen presenting cells (APC) include macrophages (Mφ) and dendritic cells. While macrophages that are capable of presenting antigens to the lymphocytes are rarely found in the intra-epithelial compartment, they constitute 5–10% of the total PP cells.

The lymphoid tissues also contain many post-capillary, high endothelial venules where lymphocytes emigrate, especially but not exclusively from other mucosa sites.

The next location of lymphoid tissue is the regional lymph nodes, including mesenteric lymph nodes (mLN) and bronchiotracheal lymph nodes. They are located along the major blood vessels and have efferent lymphatic vessels leading sequentially into the cisterna chyli and the thoracic duct. The regional lymph nodes located in other mucosal tissues also ultimately drain into the thoracic duct.

Other components of the mucosal immune system consist of a population of mast cells with peculiar characteristics, scattered in the intestinal interstitium (Goodacre *et al.*, 1979). These play a significant role in the development of allergic symptoms to food allergens from homocytotropic antibodies.

Functional interaction between mucosal and systemic immune responses

The mucosal and systemic immune systems could be regarded as independent but closely interrelated entities, each with its own compartmentalization and specialization. The mucosal immunity, although essentially similar to the systemic system in terms of afferent, efferent and regulatory components, has developed certain peculiar characteristics and adaptive mechanisms for responding to the foreign antigens to which it is constantly exposed. It is presented with different ranges of antigens and produce different subtypes of antibodies. While the mucosal system produces predominantly but not exclusively secretory IgA (sIgA), the systemic system produces predominantly IgG (Table 1a).

Both of them acting independently are essential for protecting the host from infections on one hand, and from undesirable immunological reaction to innocuous environmental antigens on the other hand. They are constantly exchanging immunological messages and tend to complement each other in their respective responses. While the mucosal system responds mainly to locally presented antigens, it is also capable of responding to systemic antigens that might be transported to it through the blood circulation (Ogra *et al.*, 1984). The systemic immune system might also mount immune responses to mucosally presented

Table 1 (a) Major differences between mucosal and systemic immune responses, (b) Mucosal immune responses at different stages of antigen arrest

	Mucosal immunity		**Systemic immunity**	
(a)				
Antigenic exposure	Both potentially harmful and essentially life-saving		Mainly harmful foreign antigens	
Regulatory mechanisms	Potent immunosuppressive mechanisms		Minimal immunosuppressive mechanisms	
Antibody response	Predominantly sIgA		Predominantly IgG	
(b)				
Stage of antigen trapping	**Intra-epithelial cells**	**Submucosal nodules**	**Peyer's patches**	**Mesenteric nodes**
Soluble antigens	–	++	+	++
Particulate proliferative antigen	+	+	++	+
Intracellular parasites	++	±	+	±
Cytotoxicity	++	+	+	+
Humoral responses-antibodies	–	+	++	+
Specific immunosuppression	–	++	+	++

++ Predominant function; + Subsidiary function.

antigens, which escape the local responses and find their way into the circulation (Friedman, 1996). A proportion of the lymphocytes primed at the mucosal site migrate into the general circulation, and vice versa. For example, the mean proportion of IgG producing lymphocytes in the rabbit mammary glands were increased after both intravenous immunization and mucosal presentation of similar antigens, although their mean level was higher after intravenous immunization (Peri *et al*., 1982).

Mechanisms of mucosal immune responses and systemic tolerance

The mucosa is continuously coming into contact with a wide range of environmental antigens, some of which are potentially harmful, such as pathogenic micro-organisms, others beneficial to the host, such as the normal intestinal flora, as well as those that are essential for life, such as food antigens. The mucosal surface must have developed mechanisms by which it is able to identify the relative 'harmfulness' of each antigen and be able to mount appropriate immunological responses, ranging from immune tolerance, through immune suppression to specific cellular or humoral immune reactivity. The degree of invasiveness or ability to penetrate intact mucosal surface might be a possible mechanism accounting for the overall adaptation of the mucosa. This alone would, however, not account for the overall specificity of mucosa responses, as most of the macromolecular foreign antigens coming into contact with the epithelial surface, including food materials, are transported across the mucosa to varying degrees by the specialized M cells.

The immune system at the mucosal level could be regarded as consisting of a functionally hierarchical arrest-points or immunological stations, through which absorbed antigens have to go through. They thereby elicit various immunological responses, depending on the degree of their 'aggressiveness' or potential danger to the organism, in line with the proposed 'Danger' model of immune reactivity (Matzinger, 1994). The nature of the immune response could also be related to the pattern of migration of the antigens across the different immunological regions, each of which tend to induce a predominantly peculiar response (Table 1b).

Oral tolerance (OT) is the phenomenon of systemic unresponsiveness to parenteral immunization following oral presentation of the antigen. A similar process has also been observed following nasal mucosal presentation of antigens, and seems to buttress the concept of a 'common mucosal immunity' (Rosales *et al*., 1984; Waldo *et al*., 1994). The characteristics of tolerogenic antigens, consisting of soluble or particulate proteins, in contrast to proteins with lectin or 'lectin-like' binding properties, have been recognized (de Aizpurua & Russell-Jones, 1988). For example, it was observed that the cells of the murine PP showed little or no proliferative response to ovalbumin (OVA) in contrast to active proliferation induced by *Streptococcus mutans*, both orally presented in appropriate dosage. This lack of proliferative response to OVA by the cells was observed despite the evidence of an increased level of specific systemic tolerance to OVA compared with *S. mutans* antigen (Challacombe & Tomasi, 1980).

The intra-epithelial lymphocytes constitute the first line of the local immune system coming into contact with luminal antigens. They are probably adapted to recognize and respond mainly to the 'dangerous' antigens, while allowing the more innocuous ones to proceed to the next site of immunological reactivity. Their predominance of cytotoxic and suppressor T cell populations suggests that they are particularly suited to respond to rapidly proliferating intracellular pathogens, such as viruses.

Other sites of immunological surveillance consists of the isolated sub-epidermal nodules of lymphoid cells and aggregated follicles in the LP (GALT). There is evidence that the site of primary immunological response as well as the amplification of IgA production by B cells that were primed outside the mucosa is at the level of aggregated lymphoid tissues, including the PP. At this stage, the 'highly virulent' antigens, such as rapidly multiplying particulate antigens are arrested and they sensitize the resident lymphocytes to multiply and produce antibodies against the offending antigens. The production of the sIgA is particularly favoured at this site, as well as all other immunoglobulin isotypes. Sensitized lymphocytes produced at this site migrate to other mucosal

surfaces to mediate similar humoral responses, as well as seeding into the systemic lymphoid organs, including the spleen, to mediate a similar systemic immune response (Cebra *et al*., 1984). This adoptive systemic humoral response is, however, less effective and quantitatively incomparable with the usual response that would have been induced if the antigen was directly introduced into the systemic circulation. This might account for the relatively low levels of systemic antibodies against common respiratory pathogens such as following *Hemophilus influenza* type B infection or immunization under 2 years of age (Rosales *et al*., 1984). On the other hand, soluble, non-proliferating antigens, such as food macromolecules or soluble products of bacterial intestinal flora, are immunologically regarded as 'innocuous' or 'beneficial' and are allowed to pass through this stage, eliciting a less aggressive but not ineffective immune reaction.

The LP might also represent a favoured site for the induction of OT. This could be attributed to a peculiar functional characteristics of its APCs, including a preferential activation of CD8+ T lymphocytes (Harper *et al*., 1996). This would suggest that the primary immune responses elicited against absorbed food molecules would be predominantly specific tolerance or hyporesponsiveness, as the LP represents a much greater total surface area for absorption compared with the aggregated lymphoid tissues, including the PP.

This proposition is further strengthened by the recent discovery of a mechanism involving inducible cyclo-oxygenase-2-dependent arachidonic acid metabolites, particularly prostaglandin E_2 (PGE_2) in LP MNC, mainly macrophages. These metabolites are potent modulators of unwanted immune reactions against dietary and non-pathologic intestinal antigens (Newberry *et al*., 1999), in conjunction with the T-helper cells and their cytokines.

The next site of immunological response consists of mLN, which receive predominantly 'safe' soluble antigens, which more readily escape from being trapped at the sub-epithelial level. This site is particularly adapted to stimulate specific immune suppressive responses, particularly the suppression of IgE production, and prevents unwanted reactions against essential life-saving foreign antigens. Although suppressor T cells are also found in the PP (Asherson *et al*., 1977), they are probably quantitatively less significant than those produced in the mLN (Challacombe & Tomasi, 1980). The mLN consist of relatively greater proportion of T lymphocytes. In contrast to the cells obtained from the PP, mNL cells showed reduced proliferative responses to *S. mutans*, compared with their response to OVA. Furthermore, this study also showed that only cells of mNL could mediate adoptive transfer of systemic tolerance, rather than PP cells (Challacombe & Tomasi, 1980).

The sensitized cells transfer the suppressive immunological responses into the systemic circulation. These cells would mediate, for example, the observed tolerance to orally presented antigens (Cebra *et al*., 1984). The significance of a temporally delayed suppression of allergy-inducing IgE was observed clinically in children with respiratory syncytial virus (RSV) infection. Cell-bound IgE could be detected on the respiratory epithelial cells of all the patients at the onset of the infection and then disappeared in most cases. The IgE antibodies only persisted in children who later developed allergic responses, bronchiolitis and bronchospasm, suggesting an intrinsic defect in late-stage IgE suppression (Welliver *et al*., 1981). This seems to suggest that the earliest manifestations of immune reactivity to the mucosaly-presented antigen consist predominantly of local humoral responses, followed later by an induction of immunosuppression and systemic tolerance.

Some of the soluble, non-proliferative antigens, being relatively innocuous, could still escape being trapped at the mLN level and enter into the general circulation. They then induce a systemic response, consisting of predominantly IgG antibody response. Such antigens are also able to induce a peculiar form of systemic tolerance, apparently because of their presentation to systemic lymphocytes in the absence of co-stimulatory molecules (Friedman, 1996).

The exact T-cell mechanism involved in induction of OT differs according to the dosage and frequency of the tolerogenic antigen, and the age at antigenic challenge (Weiner *et al*., 1994; Moreau & Gaboriau-Routhiau, 1996). Low doses generally induce preferentially either Th1 or Th2 responses with the secretion of suppressive cytokines, such as transforming growth factor-β (TGF-β) and interleukins (IL)-4, IL-10, granulocyte-macrophage

colony stimulating factor (GM-CSF) and interferon-gamma (IFN-γ) (Hoyne *et al*., 1993), while larger doses induce extrathymic anergy (Lundin *et al*., 1996) or depletion (Chen *et al*., 1995) of the T cells. The Th2 responses seem to be more pronounced and more persistent than the Th1 responses (Kang *et al*., 1999). The ability of cytokines and other neuro-endocrine factors abundantly present in human milk, to modulate the induction of OT provides a plausible explanation for the protective roles of breastfeeding in the early neonatal period. Production of IL-4 is enhanced and IFN-γ is abnormally defective in children with inflammatory atopic diseases such as asthma and allergic dermatitis, while isolated IL-4 enhancement occurs in IgE-mediated allergies (Campbell *et al*., 1998).

A beneficial soluble antigen, which normally should elicit principally immune tolerance at the sub-epithelial immune tissues, might fail to do so, either through abnormal metabolism, or through an abnormal accumulation at the sub-epithelial space, thereby leading to the development of undesirable immunological responses such as allergy. An allergic reaction could also develop against a normally formed and transported antigen, if the host has deficient immuno-suppressive responses. This seems to be the case in a number of atopic children who were found to have a defective total count and sub-populations of T lymphocytes. On the other hand, if a soluble antigen bypasses all the stages of mucosal immune system and is transported directly into the systemic circulation, which is not adapted to inducing effective immuno-suppressive reactions, it could lead to an active immune response, which might be potentially life-threatening. Allergic individuals have increased propensity for forming immune complexes of IgG and IgE after challenge with food particles, compared with normal individuals, who produce more IgA- and rarely IgE-complexes (Juto, 1980; Weaver *et al*., 1987). This might be a reflection of increased intestinal permeability of these individuals to food antigens (Soothill, 1984).

Peculiar characteristics of neonatal immunity

The immune system of the newborn is essentially similar to that of the adult, but functionally immature. They are only capable of ineffective immune responses, which seem to have been falsely generally accepted as a propensity for developing immune tolerance. The 'immaturity' of their immune system or the so called 'immuno-deficiency of immaturity' is related to a number of factors. These include the presence of a reduced number of antigen-presenting cells relative to T lymphocytes, the majority of T lymphocytes being phenotypically and functionally 'naive', decreased ability of B lymphocytes to produce antibodies against bacterial polysaccharide antigens and reduction in serum complement activity (Ridge *et al*., 1996; Schelonka & Infante, 1998).

The newborn is also characterized by a high spontaneous suppressor T cell activity, impaired NK cell activity, sub-optimal macrophage and polymorphonuclear cell functions. Other peculiar characteristics of the newborn immunity include paucity of serum IgA and sIgA in external secretion during the first few days of life which gradually increase to adult levels over an average of 12 years and 5 weeks, respectively (Quie, 1990). This period on the other hand, also corresponds with a sudden exposure to a multiplicity of extra-uterine environmental antigens, many of which could elicit potentially harmful local and systemic immune reactions. Serious life-threatening neonatal infections are caused by microbial species that are infrequently associated with infections in adults (Wolach *et al*., 1994). Although the number of neutrophils in the circulation is high in the normal neonate, their bone marrow pool is limited. T lymphocytes are present in normal numbers, but their responses to antigens are somewhat slower than in adult cells. Specific anti-microbial antibody production by the newborn infant B lymphocytes is delayed, their maturation into antibody-producing plasma cells occurring gradually during the first weeks of life (Quie, 1990). Newborns also have an impaired complement activity as compared with adults and significantly reduced complement components except for C7 (Mills *et al*., 1979). Nonspecific defence mechanisms such as gastric acid and peptic secretions, and biliary salt concentration, are also not fully developed during the first few weeks of life.

However, neonates have protective antibodies against many microbes at birth provided by their

mothers via placental transfer of IgG, but these are insufficient in premature infants and they gradually disappear from the circulation as a result of being metabolized. The immunological response in neonates is primed towards Th2 cytokines and IgE production, which gradually reverts back to normal adult response type with increasing age (Cummins & Thompson, 1997; Prescott *et al*., 1998). Hence infants are more susceptible to eliciting allergic reactions to foreign antigens in the absence of specific IgE suppressor, compared with the adults. Neonates are also capable of developing auto-antibodies against insulin and IgE antibodies against β-lactoglobulin, major causes of childhood diabetes and food allergy, respectively (Vaarala *et al*., 1999; Kato *et al*., 1999).

Mechanisms of breast-milk protection against immunological diseases

The natural ability of the host to be free from unwanted sensitization to essential or harmless foreign antigens, continuously exposed to it at the mucosal interfaces, must depend on its repertoire of immuno-suppressive mechanisms, particularly against IgE production. This mechanism seems to be concentrated at the level of sub-epithelial lymphoid tissues and the regional lymph nodes such as the mNL (Jarrett, 1984). The mediators of this suppressive role are predominantly a sub-set of T lymphocytes located in these lymphoid organs and are mediated systemically both by migrating T lymphocytes as well as through their soluble products. Both Th1 and Th2 mechanisms are involved in the induction of OT (Kang *et al*., 1999).

The mammary gland also represents a site of mucosal immunity and possesses sessile activated suppressive and stimulatory lymphocytes that have migrated from other mucosal sites. Some of these cells eventually find their way into the milk secretion and are transported to the suckling infant. The profile of lymphocytes present in the breast-milk seems to reflect accurately the selective adaptation of the mucosal immune system to different antigens. The proportion of T lymphocytes bearing markers of recent activation are significantly increased in the milk, showing a range of antigen reactivities which differ from those in the blood (Gibson *et al*., 1991; Wirt *et al*., 1992). Most of the milk lymphocytes also consist of the γδ T lymphocytes, as well as a relatively large proportion of histamine H_2 receptor-bearing T lymphocytes. Both lymphocytes are known to suppress specific antibody production. Breastmilk also contain a number of soluble components known to suppress lymphocyte activities (Schnitzler *et al*., 1982; Bertotto *et al*., 1991). It is interesting to note that γδ T lymphocytes have been shown to be essential for the induction and maintenance of systemic tolerance to oral antigens, at least in experimental animals (Mengel *et al*., 1995; Gieseler, 1996).

There are at least 100 nutritive and antimicrobial components of human milk that are either absent or present in only insignificant quantities in the cow's milk formula (Table 2a). These include antigen-specific sIgA, lysozyme, immunocompetent cells, long-chain unsaturated fatty acids, lactoferrin and essential amino acids, such as taurine (Table 2a).

Breastmilk also contain several soluble components that are known to be immunomodulators *in-vitro*. These include cytokines-Interleukin-1-beta (IL-1ß), Interleukin-6 (IL-6), tumour necrosis factor (TNF)-α and TGF-β; nucleotides, anti-idiotypic antibodies, α-tocopherol, β-casomorphins and prolactin. The importance of these breastmilk components have been demonstrated mainly through animal experiments, where certain milk hormones, including epithelial growth factors (EGF) and prostaglandins, have been shown to be absorbed into the circulation and capable of modulating the immune responses (Goldman, 1993).

Breastmilk also contain several defence factors that protect the infant by non-inflammatory mechanisms. In addition to this, it contains a wide range of anti-inflammatory components, which protect the delicate intestinal tissues against damaging inflammatory reactions (Goldman, 1993).

Breastfeeding is capable of protecting the infant against the development of common infectious diseases, autoimmune disorders and allergic diseases through a number of mechanisms. (i) First, it offers protection to the infant and its immune system against sensitization to allergens, at a susceptible period when such a sensitization might lead to allergy rather than tolerance, by

Table 2 (a) Functions of major defence factors in human milk and comparison of levels in cow's milk, (b) Humoral and cellular protective components of the human breast milk

Anti-microbial factors	Human milk	Cow's milk-based formula	Functions
(a)			
sIgA	0.2 g dL^{-1}	Absent specificity	Antigen binding
Oligosaccharides	1.2 g dL^{-1}	0.2 g dL^{-1}	Receptor analogues
Lysozyme	50–250 μg mL^{-1}	↓↓	Degrade peptidoglycans, bacteriostatic
Lactoferrin	0.2 g dL^{-1}	Trace	Iron chelation, bacteriostatic
Complement proteins	Active	Denatured/inactive	Opsonins, viral neutralization, bacteriolysis
Active milk cells	++	Killed	Immune priming/modulation
MFGM	++	Destroyed	Bind bacteria, ?stimulate milk cells
β-Lactose	++	↓↓	Favourable gut flora
Epithelial growth factors	++	Absent in formulas	Gut maturation
(b)			
	Humoral	**Cellular**	
Specific	Immunoglobulins sIgA, IgG, IgM, IgD and IgE	T- and B-lymphocytes	
Nonspecific	Lactoferrin	Macrophages	
	Lysozyme	Neutrophils	
	Lipoprotein lipase	Epithelial cells	
	Bifidus factor/ β-lactose		
	Complement proteins		
	Neuramin		
	Casein		
	Milk lipids		
	MFGM*		
	Glycoproteins		
	Free oligosaccharides		
	Nucleotides		
	Cytokines		
	Other immuno-modulators		
	Epithelial growth factors		
	Anti-inflammatory factors		
	Anti-oxidants		
	Cyto-protective factors		

*Milk fat globule membrane.

eliminating such antigens from the infant's diet. (ii) Secondly, colostrum and breast-milk simultaneously supply numerous products of passive immunity at the mucosal surface, while the infant's immunity is still maturing. (iii) Thirdly, breastfeeding modulates the immune responses of the infant in such a way that it encourages immunosuppression of antibody production to harmless antigens, particularly the suppression of IgE isotype, and (iv) it actively primes and stimulates the neonatal immune responses to harmful pathogens. (v) It also enhances intestinal mucosal maturation, (vi) possess many anti-inflammatory factors and mechanisms and (vii) provides many long-term favourable immune priming advantages.

Passive immunity and reduced exposure to antigens by early breastfeeding

The main role of breast-milk antibodies which are predominantly secretory IgA (sIgA), directed against many food and pathogenic antigens is to bind these antigens in the intestine and prevent their attachment to the gut epithelium. Breastfeeding thereby protects against specific pathogens including *Salmonella*, *Vibrio cholerae*, *Escherichia coli*, *Shigella*, polioviruses, rotavirus and respiratory

syncytial virus (Goldman, 1993). Human milk sIgA is secreted by activated B lymphocytes in the maternal PP, after undergoing a TGF-β-mediated isotope switching from IgM + to IgA + cells, and migrating into the blood circulation. They then home into the mammary gland, (as well as into respiratory tract and other mucosal sites) under influences of certain hormones and poorly understood mechanisms.

A deficient secretion of sIgA in the breast milk has been associated with an increased risk of developing atopic diseases in the nursing infants (Savilahti *et al*., 1991). In rats, maternal anti egg-albumin IgG transferred to the offspring via milk is capable of suppressing IgE response to the same antigen on subsequent challenge (Jarrett & Hall, 1986).

β-Lactoglobulin, a major cause of food-related allergies is a normal component of cow's milk, but is usually absent in human milk. When present in smaller quantities in certain human milk samples, as a result of maternal ingestion of cow's milk and producing allergic symptoms, it can easily be controlled by the elimination of cow's milk from the mother's diet (Sorva *et al*., 1994). The presentation of excessive levels and multiple doses of β-lactoglobulin in cow's milk to the formula-fed infant might account for its propensity to suppress the induction of tolerance, induce systemic and local immunity in the children, compared with the low doses present in human milk (Garg *et al*., 1999). The several immunomodulating factors in human milk would also prevent the induction of IgE antibodies against the low concentrations of the allergens that might be present in the mother's milk. The observed protection of breastfeeding against the development of type 1 diabetes in childhood has been partly ascribed to the elimination of inductive auto-antigens in the diet during the susceptible neonatal period (Vaarala *et al*., 1999).

Other passive defence factors in human milk include an array of oligosaccharides and glycoconjugates acting as receptor analogues that inhibit the binding of certain enteric and respiratory pathogens and their toxins (Table 2b). Milk fat globule membrane (MFGM) mucin components and milk casein are also known to inhibit the adherence of common neonatal pathogens including S-fimbriated *E. coli*, *Streptococcus pneumoniae* and *H. influenzae*, as well as defending against rotavirus infection (Aniansson *et al*., 1990; Schroten *et al*., 1992; Goldman, 1993).

Unfavourable intestinal flora in formula-fed infants, comprising predominantly of gram-negative organisms, could also act as adjuvants to food antigens and enhance an abnormal sensitization and allergic reaction to these antigens. On the other hand, breast milk contains bifid factors which favour the colonization of the intestinal tract by *Lactobacillus* rather than *Enterobacteriae* and other potentially pathogenic organisms (Duffy *et al*., 1994).

Active priming and stimulation by breastfeeding

Human breast milk and colostrum also contain a number of soluble factors that stimulate local immune responses to foreign antigens (Juto, 1985; Mincheva-Nilsson *et al*., 1990). Among the immunostimulators are specific IgA helper stimulating factors and soluble CD23. Soluble CD23 has a double beneficial effect of both inhibiting allergic (IgE) immune responses while activating resting lymphocytes through its ability to stimulate monokine synthesis from activated monocytes in the absence of antigens (Sarfati *et al*., 1986). The recent discoveries of a wide range of biologically active cytokines in human milk further suggest a physiological role of breast milk in modulating the immune responses of the recipient infant (Goldman *et al*., 1996). Their postulated functions include T lymphocyte activation, enhanced IgA and secretory component (SC) production, and isotype switching to IgA^+ B-lymphocytes.

The immunomodulatory effect of breast milk is not only limited to the intestinal mucosal system, but it also influences specific immunity in other distant mucosal sites. Increased levels of certain immunological factors have been reported in breastfed infants which could not be accounted for by passive transfer from the milk. Breastfed infants secrete increased amounts of IgA in the intestinal tract and other external secretions including the urine, not accounted for by the level of sIgA in the breast milk, thereby mounting a protective response to mucosal infections (James-Ellison *et al*., 1997). Other yet unidentified components of human milk are also capable of

stimulating local intestinal sIgA synthesis, in breastfed infants (Koutras & Vigorita, 1989). Treatment of children with lysozyme, which is abundant in breast milk was shown to increase sIgA secretion in the intestine (Lodinova & Jouja, 1977).

Breast milk also seems to influence the systemic immunity, in such a way that it offers increased protection to the breastfed infant. The appearance of increasing serum IgA is delayed in the breastfed infants compared with the formula fed infants. Breastfeeding stimulates an enhanced production of α-interferon in the circulation of infants with RSV infection (Chiba *et al*., 1987). Similar increments in the levels of circulating fibronectin in breastfed infants, not accounted for by the levels in the breast milk, is another pointer to the systemic immunostimulatory functions of breastfeeding (Friss *et al*., 1988). The complement system function levels is also increased in breastfed infants (Barriga *et al*., 1995).

Milk T and B lymphocytes have been able to transfer priming to the breastfed offspring in some experimental models. This together with transfer of numerous cytokines and growth factors via milk may explain why breastfed the infant might respond better to both infections and vaccines (Hanson, 1998).

Immunosuppression by breastfeeding

The GALT in infancy has been shown to contain more IgE-bearing or IgE-precursor cells compared with the adult. Hence infants are more susceptible to eliciting allergic reactions to foreign antigens in the absence of specific IgE suppressors, compared with the adults. Several specific factors have been detected in colostrum and breast milk which are inhibitory to immunoproliferative responses of lymphocytes towards mitogens (Crago & Mestecky,1983; Horne *et al*., 1983; Mandalapu *et al*., 1995). These may be helpful to the infant by suppressing the induction of potentially harmful immunological reactions, while simultaneously supplying the infant with products of passive immunity until the infant's immune system is more mature and able to respond to these antigens appropriately.

Infants on breast milk usually tend to have lower levels of total serum IgE than those fed on cow's milk for up to 4 months of age, even after exclusive milk feeding is ended. This difference could not be accounted for by the presence of IgE antibodies in cow's milk, as no such antibodies could be detected (Juto, 1980; Sasai *et al*., 1994). The IgE binding factor, a known inhibitor of ongoing IgE synthesis in pre-activated B lymphocytes is present in the human colostrum, and probably significantly contribute to the reduced incidence of atopic diseases among breastfed infants (Sarfati & Delespesse, 1988).

Prostaglandin E2 that is present in large quantities in breast milk (up to 100-fold higher than in the blood) is known to inhibit the production of IgE by the peripheral blood mononuclear leucocytes from atopic children *in vitro*.

β-Lactoglobulin that is present in cow's milk and is responsible for many cases of childhood food-related allergies, is capable of inducing OT in animals (Kato *et al*., 1999). Its failure to induce OT in neonates fed with cow's milk might be related to the poor immunological environment represented by cow's milk components, compared with human milk. Early inadvertent exposure to cow's milk in infants subsequently breastfed exclusively have been shown to offer long-term protection to the infants, rather than predispose them to the development of allergic diseases (Host *et al*., 1988). This further proves that human milk could serve as an immunological adjuvant, which enhances the production of immune tolerance to allergenic substances presented to the infant's gut, thereby suppressing the induction of allergic responses.

Immunomodulation by breastmilk could also be accounted for by cellular mechanisms. Most of the milk lymphocytes consist of the γδ T lymphocytes, as well as a relatively large proportion of histamine H_2 receptor-bearing T lymphocytes, both of which are known to suppress specific antibody production (Schnitzler *et al*., 1982; Gieseler, 1996). They might be capable of being transferred into the systemic circulation of the breastfed infant and suppress their specific immunity to food and other allergens. The activity of these cells might account for the reduced levels of systemic IgA, IgM and IgG in breastfed infants compared with those fed cow's milk formula (Savilahti *et al*., 1991).

It has been speculated that increased prevalence of type 1 diabetes among formula fed infants might be attributed to impaired mucosal immune functions in infants who were not exposed to the protective roles of early breastfeeding (Mayer *et al.*, 1988; Harrison & Honeyman, 1999). Breastfeeding is known to modulate development of autoantibodies against insulin in infants who are at high genetic risks of developing type 1 diabetes, thereby preventing progression into autoaggressive immunological disease (Vaarala *et al.*, 1995).

Intestinal maturation and anti-inflammatory mechanisms

A proportion of the ingested food macromolecules is known to be transported across the intestinal mucosa into the circulation. This uptake gradually diminishes with age in normal infants but might be exaggerated in food-allergic individuals and cow's milk fed infants. It is, however, not clear whether this is one of the fundamental defects leading to the development of allergic disorder or is an outcome of the disease process (Weaver *et al.*, 1987). Human milk contains many growth factors including lactoferrin, cortisol, EGF and polyamines, as well as a host of other hormones, which enhance the physiochemical maturation of the intestinal mucosal barrier against absorption of allergic macromolecules.

Many anti-inflammatory components are present in human milk and prevent induction of tissue destructive inflammatory responses in the delicate neonatal gut and might be responsible for the long-term protection against the development of certain chronic inflammatory bowel disorders. These factors include prostaglandins, EGF, cortisol, lysozyme, sIgA and anti-oxidants such as uric acid, α-tocopherol, β-carotene and ascorbic acid. In a retrospective case–controlled study, history of 'ever breastfeeding' was found to be associated with a reduced risk of developing Crohn's disease in childhood. This protective effect might also be related to the ability of breast milk to reduce the risk or severity of yet unidentified childhood infections, which trigger abnormal immunological responses in the intestine of the newborn (Whorwell *et al.*, 1979; Koletzko *et al.*, 1989).

Long- and short-term effects of early breastfeeding in modulating the risk of childhood diseases

It has long been observed that avoidance of cow's milk and other potential allergens in the first months of life reduces the incidence of allergic diseases in later childhood, irrespective of the family history of atopy (Saarinen *et al.*, 1979). This protection could even be further enhanced by restricting maternal diet to no more than 200 dL of cow milk per day, no more than one egg per week, and no tomato, fish, shellfish, nuts or foods allergenic to the mother (Chandra *et al.*, 1986; Bardare *et al.*, 1993). Feeding of any form of whole-protein infant formula has been shown to induce a sensitization of the infant as early as 4 days of life, with a significant increase in IgE levels between cord and 4th day blood in soy-fed and adapted-formula-fed babies. This increase did not occur in neonates fed breast milk and serum protein hydrolysate (Buonocore *et al.*, 1992).

Early exposure to cows' milk could increase the risk of a wide range of allergic reactions, especially in infants with a family history of atopy, including eczema, recurrent wheezing, elevated serum IgE-antibodies to cow's milk, complement activation *in vivo* after milk challenge and haemagglutinating antibodies to β-lactoglobulin (Chandra, 1979). Breastfeeding also offers long-term protection against the development of asthma and bronchitis, both in infants from allergic and non-allergic families (Hide & Guyer, 1981). Exclusively breastfeeding for a minimum of 6 weeks could lead to a significant reduction in the incidence of these allergic diseases.

Breastfeeding has also been shown to prevent up to half the incidence of diarrhoeal illness and dehydration among infants, also reducing the incidence of any otitis media by 19%, and particularly reducing prolonged episodes by up to 80%, within the first and second year of life, as well as reducing the incidence of upper respiratory tract infections and pneumonia (Jason *et al.*, 1984; Dewey *et al.*, 1995).

There is evidence that the immunomodulatory effect of breastfeeding on the infantile immune response persist for several years, even long after cessation of exclusive breastfeeding. The breastfed

infants have a reduced tendency to develop clinical allergies such as atopic eczema, food and respiratory allergies, both in later childhood and adolescence, compared with the cow's or soy milk-based formula-fed infants (Gruskay, 1982). These findings have been confirmed in prolonged follow-up studies conducted over up to a 17-year period (Saarinen & Kajosaari, 1995).

The modulatory effect of breastfeeding is also evident from the subsequent clinical response of breastfed infants to maternal and sibling-donor organ transplantation. Breastfed patients often show dramatic improvements in graft function rates and a more favourable post-transplant course, as measured by the percentage of patients who had no rejection episodes during the first post-transplant year, compared with their non-breastfed counterparts (Campbell *et al*., 1984; Kois *et al*., 1984).

The EGF and similar growth factors might account for the increased size of the thymus in breastfed infants (Hasselbalch *et al*., 1996). This might possibly lead to a more advanced T lymphocyte differentiation and maturation, and consequently reduced risk of self-induced autoimmune diseases in the breastfed infants, such as insulin-dependent diabetes mellitus and Crohn's disease.

Conclusion

There are many possible mechanisms by which early breastfeeding could modulate the development of food and other mucosal allergies in the newborn, especially among those with high risk factors for atopy. Despite a number of studies reporting negative or non-conclusive effects, the greater weight of evidence supports the fact that breastfeeding protects the child against allergic and other immunological disorders, including type 1 diabetes mellitus, lymphomas and chronic inflammatory bowel disorders. With the exception of a few cases now reported in some Western countries, exclusive breastfeeding provides a practicable means of treating allergy-related symptoms by strict elimination of the offending antigens from the mothers diet. Other allergic children could also be effectively treated with the so called hypoallergenic (protein hydrolysate) milk formulas while the resistant cases would only benefit from amino acids-based infant formulae.

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References

de Aizpurua, H.J. & Russell-Jones, G.J. (1988). Oral vaccination. Identification of classes of protens that provoke an immune response upon oral feeding. *Journal of Experiemental Medicine*, **167**, 440–451.

Aniansson, G., Andersson, B., Lindstedt, R. & Svanborg, C. (1990). Anti-adhesive activity of human casein against *Streptococcus pneumoniae* and *Haemophilus influenzae*. *Microbiology and Pathology*, **8**, 315–323.

Asherson, G.L., Zembala, M., Perera, M.A., Mayhew, B. & Thomas, W.R. (1977). Production of immunity and unresponsiveness in the mouse by feeding contact sensitizing agents and the role of suppressor cells in the peyer's patches, mesenteric lymph nodes and other lymphoid tissues. *Cellular Immunology*, **33**, 145–155.

Bardare, M., Vaccari, A., Allievi, E. *et al*. (1993). Influence of dietary manipulation on incidence of atopic disease in infants at risk. *Annals of Allergy*, **71**, 366–371.

Barriga, C., Pombero, I., Duran, J., Forner, A., Cardesa, J. & Rodriguez, A.B. (1995). Serum hemolytic and bactericidal activity in breast and formula-fed infants. *Revue Espanol Fisiologie*, **51**, 213–218.

Bertotto, A., Gerli, R., Castellucci, G., Scalise, F. & Vaccaro, R. (1991). Human milk lymphocytes bearing the gamma/delta T-cell receptor are mostly delta TCS1-positive cells. *Immunology*, **74**, 360–361.

Bienenstock, J., Befus, A.D., McDermott, M., Mirski, S., Rosenthal, K. & Tagliabue, A. (1983). The mucosal immunological network: compartmentalization of lymphocytes, natural killer cells, and mast cells. *Annals of New York Academy of Science*, **409**, 164–170.

Bjorksten, B. (1984). (IgE and lymphocyte function in small children as allergy predictors) IgE und Lymphozytenfunktion bei kleinkindern als Vorhersager einer Allergie. *Zeitschrifft Fuer Hautkrankheiten*, **59**, 817–825.

Bockman, D.E., Boydston, W.R. & Beezhold, D.H. (1983). The role of epithelial cells in gut-associated immune reactivity. *Annals of New York Academy of Science*, **409**, 129–144.

Buonocore, G., Zani, S., Tomasini, B., Tripodi, V., Grano, S. & Bracci, R. (1992). Serum IgE concentrations in the neonatal period. *Biology of Neonate*, **62**, 10–14.

Businco, L., Cantani, A., Longhi, M.A. & Giampietro, P.G. (1989). Anaphylactic reactions to a cow's milk whey protein hydrolysate (Alfa-Re, Nestle) in infants with cow's milk allergy. *Annals of Allergy*, **62**, 333–335.

Businco, L., Lucenti, P., Arcese, G., Ziruolo, G. & Cantani, A. (1994). Immunogenicity of a so-called hypoallergenic formula in at-risk babies: two case reports. *Clinical and Experimental Allergy*, **24**, 42–45.

Campbell, D.A. Jr, Lorber, M.I., Sweeton, J.C., Turcotte, J.G., Niederhuber, J.E. & Beer, A.E. (1984). Breastfeeding and maternal-donor renal allografts. Possibly the original donor-specific transfusion. *Transplantation*, **37**, 340–344.

Campbell, D.E., Hill, D.J. & Kemp, A.S. (1998). Enhanced IL-4 but normal interferon-gamma production in children with isolated IgE mediated food hypersensitivity. *Pediatric Allergy and Immunology*, **9**, 68–72.

Cebra, J.J., Fuhrman, J.A., Griffin, P., Rose, F.V., Schweitzer, P.A. & Zimmerman, D. (1984). Changes in specific B cells and the dissemination of the primed state in vivo following antigenic stimulation by different mucosal routes. *Annals of Allergy*, **53**, 541–549.

Challacombe, S.J. & Tomasi, T.B. Jr (1980). Systemic tolerance and secretory immunity after oral immunization. *Journal of Experiemental Medicine*, **152**, 1459–1472.

Chandra, R.K. (1979). Prospective studies of the effect of breastfeeding on incidence of infection and allergy. *Acta Paediatrica Scandinavia*, **68**, 691–694.

Chandra, R.K., Puri, S., Suraiya, C. & Cheema, P.S. (1986). Influence of maternal food antigen avoidance during pregnancy and lactation on incidence of atopic eczema in infants. *Clinical Allergy*, **16,** 563–569.

Chen, Y., Inobe, J., Marks, R., Gonnella, P., Kuchroo, V.K. & Weiner, H.L. (1995). Peripheral deletion of antigen-reactive T cells in oral tolerance. *Nature*, **376**, 177–180.

Chiancone, E., Gattoni, M., Giampietro, P.G., Ragno, V. & Businco, L. (1995). Detection of undegraded beta-lactoglobulins and evaluation of the molecular weight of peptides in hydrolysate cow's milk formulae. *Journal of Investigative Allerlogy and Clinical Immunology*, **5**, 228–231.

Chiba, Y., Minagawa, T., Mito, K. *et al*. (1987). Effect of breastfeeding on responses of systemic interferon and virus-specific lymphocyte transformation in infants with respiratory syncytial virus infection. *Journal of Medical Virology*, **21**, 7–14.

Crago, S.S. & Mestecky, J. (1983). Immunoinhibitory elements in human colostrum. *Survey in Immunology Research*, **2**, 164–169.

Cummins, A.G. & Thompson, F.M. (1997). Postnatal changes in mucosal immune response: a physiological perspective of breastfeeding and weaning. *Immunology and Cell Biology*, **75**, 419–429.

Dewey, K.G., Heinig, M.J. & Nommsen-Rivers, L.A. (1995). Differences in morbidity between breastfed and formula-fed infants. *Journal of Pediatrics*, **126**, 696–702.

Duffy, L.C., Zielezny, M.A., Riepenhoff-Talty, M. *et al*. (1994). Effectiveness of Bifidobacterium bifidum in mediating the clinical course of murine rotavirus diarrhea. *Pediatrics Research*, **35**, 690–695.

Friedman, A. (1996). Induction of anergy in Th1 lymphocytes by oral tolerance. Importance of antigen dosage and frequency of feeding. *Annals of New York Academy of Science*, **778**, 103–110.

Friss, H.E., Rubin, L.G., Carsons, S., Baranowski, J. & Lipsitz, P.J. (1988). Plasma fibronectin concentrations in breastfed and formula fed neonates. *Archives of Diseases in Childhood*, **63**, 528–532.

Garg, S., Bal, V., Rath, S. & George, A. (1999). Effect of multiple antigenic exposures in the gut on oral tolerance and induction of antibacterial systemic immunity. *Infection and Immunity*, **67**, 5917–5924.

Gibson, C.E., Eglinton, B.A., Penttila, I.A. & Cummins, A.G. (1991). Phenotype and activation of milk-derived and peripheral blood lymphocytes from normal and coeliac subjects. *Immunology and Cell Biology*, **69**, 387–393.

Gieseler, R. (1996). γδ-T-Zellen und das mukosassozierte lymphoide Gewebe. Eine Hypothese zur immunologischen Funktionen der γδ-T-Zellen. Biologische Medizin. *International Journal of Biochemistry*, **5**, 204–210.

Giovannini, M., Agostoni, C., Fiocchi, A., Bellu, R., Trojan, S. & Riva, E. (1994). Antigen-reduced infant formulas versus human milk: growth and metabolic parameters in the first 6 months of life. *Journal of Americal College of Nutrition*, **13**, 357–363.

Goldman, A.S. (1993). The immune system of human milk: antimicrobial, antiinflammatory and immunomodulating properties. *Pediatrics Infectious Diseases*, **12**, 664–671.

Goldman, A.S., Chheda, S., Garofalo, R. & Schmalstieg, F.C. Jr (1996). Cytokines in human milk: properties and potential effects upon the mammary gland and the neonate. *Journal of Mammary Gland Biology and Neoplasia*, **1**, 251–258.

Goodacre, R., Davidson, R., Singal, D. & Bienenstock, J. (1979). Morphologic and functional characteristics of human intestinal lymphoid cells isolated by a mechanical technique. *Gastroenterology*, **76**, 300–308.

Gruskay, F.L. (1982). Comparison of breast, cow, and soy feedings in the prevention of onset of allergic disease: a 15-year prospective study. *Clinical Pediatrics of Philadelphia*, **21**, 486–491.

Hanson, L.A. (1998). Breastfeeding provides passive and likely long-lasting active immunity. *Annals of Allergy and Asthma Immunology*, **81**, 523–533.

Harper, H.M., Cochrane, L. & Williams, N.A. (1996). The role of small intestinal antigen-presenting cells in the induction of T-cell reactivity to soluble protein antigens: association between aberrant presentation in the lamina propria and oral tolerance. *Immunology*, **89**, 449–456.

Harrison, L.C. & Honeyman, M.C. (1999). Cow's milk and type 1 diabetes: the real debate is about mucosal immune function. *Diabetes*, **48**, 1501–1507.

Hasselbalch, H., Engelmann, M.D., Ersboll, A.K., Jeppesen, D.L. & Fleischer-Michaelsen, K. (1996). Breast-feeding influences thymic size in late infancy. *European Journal of Pediatrics*, **158**, 964–967.

Heine, R.G., Cameron, D.J., Catto-Smith, A.G., Chow, C.W., Francis, D.E. & Hosking, C.S. (2000). Role of food protein intolerance in infants with persistent distress attributed to reflux esophagitis. *Journal of Pediatrics*, **136**, 641–647.

Hide, D.W. & Guyer, B.M. (1981). Clinical manifestations of allergy related to breast and cows' milk feeding. *Archives of Diseases of Children*, **56**, 172–175.

Hill, D.J., Heine, R.G., Cameron, D.J.S., Francis, D.E.M. & Bines, B.E. (1999a). The natural history of intolerance to soy and extensively hydrolyzed formula in infants with multiple food protein intolerance. *Journal of Pediatrics*, **135**, 118–121.

Hill, D.J., Hosking, C.S. & Heine, R.G. (1999b). Clinical spectrum of food allergy in children in Australia and South-East Asia: identification and targets for treatment. *Annals of Medicine*, **31**, 272–281.

Holt, P.G. (1996). Primary allergic sensitization to environmental antigens: perinatal T cell priming as a determinant of responder phenotype in adulthood. *Journal of Experiemental Medicine*, **183**, 1297–1301.

Horne, C.H., Armstrong, S.S., Thomson, A.W. & Thompson, W.D. (1983). Detection of pregnancy associated alpha 2-glycoprotein (alpha 2-PAG), an immunosuppressive agent, in IgA producing plasma cells and in body secretions. *Clinical and Experimental Immunology*, **51**, 631–638.

Host, A., Husby, S. & Osterballe, O. (1988). A prospective study of cow's milk allergy in exclusively breast-fed infants. incidence, pathogenetic role of early inadvertent exposure to cow's milk formula, and characterization of bovine milk protein in human milk. *Acta Paediatrica Scandinavia*, **77**, 663–670.

Hoyne, G.F., Callow, M.G., Kuhlman, J. & Thomas, W.R. (1993). T-cell lymphokine response to orally administered proteins during priming and unresponsiveness. *Immunology*, **78**, 534–540.

Isolauri, E., Sutas, Y., Makinen-Kiljunen, S., Oja, S.S., Isosomppi, R. & Turjanmaa, K. (1995). Efficacy and safety of hydrolyzed cow milk and amino acid-derived formulas in infants with cow milk allergy. *Journal of Pediatrics*, **127**, 550–557.

Isolauri, E., Tahvanainen, A., Peltola, T. & Arvola, T. (1999). Breast-feeding of allergic infants. *Journal of Pediatrics*, **134**, 27–32.

James-Ellison, M.Y., Roberts, R., Verrier-Jones, K., Williams, J.D. & Topley, N. (1997). Mucosal immunity in the urinary tract: changes in sIgA, FSC and total IgA with age and in urinary tract infection. *Clinical Nephrology*, **48**, 69–78.

Jarrett, E.E. (1984). Immunoregulation of IgE responses: the role of the gut in perspective. *Annals of Allergy*, **53**, 550–556.

Jarrett, E.E. & Hall, E. (1986). Perinatal-maternal relationships in the regulation of IgE antibody responsiveness. *Journal of Allergy and Clinical Immunology*, **78**, 1000–1003.

Jason, J.M., Nieburg, P. & Marks, J.S. (1984). Mortality and infectious disease associated with infant-feeding practices in developing countries. *Paediatrics*, **74**, 702–727.

Juto, P. (1980). Elevated serum immunoglobulin E in T cell-deficient infants fed cow's milk. *Journal of Allergy and Clinical Immunology*, **66**, 402–407.

Juto, P. (1985). Human milk stimulates B cell function. *Archives of Diseases in Childhood*, **60**, 610–613.

Kang, B., Kim, K.M. & Kang, C.Y. (1999). Oral tolerance by a high dose OVA in BALB/c mice is more pronounced and persistent in Th2-mediated immune responses than in Th1 responses. *Immunobiology*, **200**, 264–276.

Kankaanpaa, P., Sutas, Y., Salminen, S., Lichtenstein, A. & Isolauri, E. (1999). Dietary fatty acids and allergy. *Annals of Medicine*, **31**, 282–287.

Kato, C., Sato, K., Eishi, Y. & Nakamura, K. (1999). The influence of initial exposure timing to beta-lactoglobulin on oral tolerance induction. *Journal of Allergy and Clinical Immunology*, **104**, 870–878.

Kelly, K.J., Lazenby, A.J., Rowe, P.C., Yardley, J.H., Perman, J.A. & Sampson, H.A. (1995). Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. *Gastroenterology*, **109**, 1503–1512.

Kois, W.E., Campbell, D.A. Jr, Lorber, M.I., Sweeton, J.C. & Dafoe, D.C. (1984). Influence of breastfeeding on subsequent reactivity to a related renal allograft. *Journal of Surgical Research*, **37**, 89–93.

Koletzko, S., Sherman, P., Corey, M., Griffiths, A. & Smith, C. (1989). Role of infant feeding practices in development of Crohn's disease in childhood. *British Medical Journal*, **298**, 1617–1618.

Koutras, A.K. & Vigorita, V.J. (1989). Fecal secretory immunoglobulin A in breast milk versus formula feeding in early infancy. *Journal of Pediatric Gastroenterology and Nutrition*, **9**, 58–61.

Lodinova, R. & Jouja, V. (1977). Influence of oral lysozyme administration on serum immunoglobulin and intestinal secretory IgA levels in infants. *Acta Paediatrica Scandinavia*, **66**, 709–712.

Lundin, B.S., Dahlgren, U.I., Hanson, L.A. & Telemo, E. (1996). Oral tolerization leads to active suppression and bystander tolerance in adult rats while anergy dominates in young rats. *Scandinavia Journal of Immunology*, **43**, 56–63.

Mandalapu, P.P., Abstract, H.F. & Paetkau, V. (1995). A novel immunosuppressive factor in human colostrum. *Cell Immunology*, **162**, 178–184.

Matzinger, P. (1994). Tolerance, dander and the extended family. *Annual Review of Immunology*, **12**, 991–1045.

Mayer, E.J., Hamman, R.F., Gay, E.C., Lezotte, D.C., Savitz, D.A. & Klingensmith, G.J. (1988). Reduced risk of IDDM among breast-fed children. *The Colorado IDDM Registry Diabetes*, **37**, 1625–1632.

Mengel, J., Cardillo, F., Aroeira, L.S., Williams, O., Russo, M. & Vaz, N.M. (1995). Anti-gamma delta T cell antibody blocks the induction and maintenance of oral tolerance to ovalbumin in mice. *Immunology Letter*, **48**, 97–102.

Mills, E.L., Bjorksten, B. & Quie, P.G. (1979). Deficient alternative complement pathway activity in newborn sera. *Pediatrics Research*, **13**, 1341–1344.

Mincheva-Nilsson, L., Hammarstrom, M.L., Juto, P. & Hammarstrom, S. (1990). Human milk contains proteins that stimulate and suppress T lymphocyte proliferation. *Clinical and Experimental Immunology*, **79**, 463–469.

Moreau, M.C. & Gaboriau-Routhiau, V. (1996). The absence of gut flora, the doses of antigen ingested and aging affect the long-term peripheral tolerance induced by ovalbumin feeding in mice. *Research in Immunology*, **147**, 49–59.

Newberry, R.D., Stenson, W.F. & Lorenz, R.G. (1999). Cyclooxygenase-2-dependent arachidonic acid metabolites are essential modulators of the intestinal immune response to dietary antigen nature medicine, **5**, 900–906.

Ogra, P.L., Welliver, R.C., Riepenhoff-Talty, M. & Faden, H.S. (1984). Interaction of mucosal immune system and infections in infancy: implications in allergy. *Annals of Allergy*, **53**, 523–534.

Peri, B.A., Theodore, C.M., Losonsky, G.A., Fishaut, J.M., Rothberg, R.M. & Ogra, P.L. (1982). Antibody content of rabbit milk and serum following inhalation or ingestion of respiratory syncytial virus and bovine serum albumin. *Clinical and Experimental Immunology*, **48**, 91–101.

Prescott, S.L., Macaubas, C., Smallacombe, T. *et al*. (1998). Reciprocal age-related patterns of allergen-specific T-cell immunity in normal vs. atopic infants. *Clinical and Experimental Allergy*, **28** (Suppl. 5), 39–44,50–1.

Quie, P.G. (1990). Antimicrobial defenses in the neonate. *Seminars in Perinatology*, **14** (4 Suppl. 1), 2–9.

Ridge, J.P., Fuchs, E.J. & Matzinger, P. (1996). Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. *Science*, **271**, 1723–1726.

Rosales, S.V., Lascolea, L.J. Jr & Ogra, P.L. (1984). Development of respiratory mucosal tolerance during *Haemophilus influenzae* type B infection in infancy. *Journal of Immunology*, **132**, 1517–1521.

Saarinen, U.M. & Kajosaari, M. (1995). Breastfeeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. *Lancet*, **346**, 1065–1069.

Saarinen, U.M., Kajosaari, M., Backman, A. & Siimes, M.A. (1979). Prolonged breast-feeding as prophylaxis for atopic disease. *Lancet*, **2**, 163–166.

Sarfati, M. & Delespesse, G. (1988). Possible role of human lymphocyte receptor for IgE (CD23) or its soluble fragments in the in vitro synthesis of human IgE. *Journal of Immunology*, **141**, 2195–2199.

Sarfati, M., Vanderbeeken, Y., Rubio-Trujillo, M., Duncan, D. & Delespesse, G. (1986). Presence of IgE suppressor factors in human colostrum. *European Journal of Immunology*, **16**, 1005–1008.

Sasai, K., Furukawa, S., Kaneko, K., Yabuta, K. & Baba, M. (1994). Fecal IgE levels in infants at 1 month of age as indicator of atopic disease. *Allergy*, **49**, 791–794.

Savilahti, E., Tainio, V.M., Salmenpera, L. *et al*. (1991). Levels of IgA and cow milk antibodies in breast milk vs. the development of atopy in children. Low colostral IgA associated with cow milk allergy. *Advances in Experimental and Medical Biology*, **310**, 417–425.

Schelonka, R.L. & Infante, A.J. (1998). Neonatal immunology. *Seminars in Perinatology*, **22**, 2–14.

Schnitzler, S., Eckert, R., Volk, D. & Gronow, R. (1982). Histamin und Immunoreaktionen. *Histaminrezeptor-Tragende Lymphozyten Allergie und Immunologie Leipzig*, **28**, 112–115.

Schroten, H., Hanisch, F.G., Plogmann, R. *et al*. (1992). Inhibition of adhesion of S-fimbriated *Escherichia coli* to buccal epithelial cells by human milk fat globule membrane components: a novel aspect of the protective function of mucins in the nonimmunoglobulin fraction. *Infection and Immunology*, **60**, 2893–2899.

Soothill, J.F. (1984). Prevention of food allergic disease. *Annals of Allergy*, **53**, 689–691.

Sorva, R., Makinen-Kiljunen, S. & Juntunen-Backman, K. (1994). Beta-lactoglobulin secretion in human milk varies widely after cow's milk ingestion in mothers of infants with cow's milk allergy. *Journal of Allergy and Immunology*, **93**, 787–792.

Strannegard, O. & Strannegard, I.L. (1999). [Why is the prevalence of allergy increasing? Changed microbial load is probably the cause] Varfor okar allergiprevalensen? Forandrad mikrobiell belastning troligaste orsaken. *Lakartidningen*, **96**, 4306–4312.

Vaarala, O., Saukkonen, T., Savilahti, E., Klemola, T. & Akerblom, H.K. (1995). Development of immune response to cow's milk proteins in infants receiving cow's milk or hydrolyzed formula. *Journal of Allergy and Immunology*, **96**, 917–923.

Vaarala, O., Knip, M., Paronen, J. *et al*. (1999). Cow's milk formula feeding induces primary immunization to insulin in infants at genetic risk for type 1 diabetes. *Diabetes*, **48**, 1389–1394.

Vanderhoof, J.A., Murray, N.D., Kaufman, S.S. *et al*. (1997). Intolerance to protein hydrolysate infant formulas: an underrecognized cause of gastrointestinal symptoms in infants. *Journal of Pediatrics*, **131**, 741–744.

Waldo, F.B., van-den-Wall, A., Bake, A.W., Mestecky, J. & Husby, S. (1994). Suppression of the immune response by nasal immunization. *Clinical Immunolology and Immunopathology*, **72**, 30–34.

Weaver, L.T., Laker, M.F., Nelson, R. & Lucas, A. (1987). Milk feeding and changes in intestinal permeability and morphology in the newborn. *Journal of Pediatric Gastroenteroloy and Nutrition*, **6**, 351–358.

Weiner, H.L., Friedman, A., Miller, A. *et al*. (1994). Oral tolerance: immunologic mechanisms and treatment of animal and human organ-specific autoimmune diseases by oral administration of autoantigens. *Annual Review in Immunology*, **12**, 809–837.

Weisselberg, B., Dayal, Y., Thompson, J.F., Doyle, M.S., Senior, B. & Grand, R.J. (1996). A lamb-meat-based formula for infants allergic to casein hydrolysate formulas. *Clinical Pediatrics of Philadelphia*, **35**, 491–495.

Welliver, R.C., Wong, D.T., Sun, M., Middleton, E. Jr, Vaughan, R.S. & Ogra, P.L. (1981). The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. *New England Journal of Medicine*, **305**, 841–846.

Whorwell, P.J., Holdstock, G., Whorwell, G.M. & Wright, R. (1979). Bottle feeding, early gastroenteritis, and inflammatory bowel disease. *British Medical Journal*, **1**, 382.

Wirt, D.P., Adkins, L.T., Palkowetz, K.H., Schmalstieg, F.C. & Goldman, A.S. (1992). Activated and memory T lymphocytes in human milk. *Cytometry*, **13**, 282–290.

Wolach, B., Carmi, D., Gilboa, S. *et al*. (1994). Some aspects of the humoral immunity and the phagocytic function in newborn infants. *Israel Journal of Medical Sciences*, **30**, 331–335.