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## Plenary Lecture

# Early nutrition and the development of immune function in the neonate

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The present review will concentrate on the development of the gut-associated lymphoid tissue and the role of early nutrition in promoting immune function. The intestine is the largest immune organ in the body, and as such is the location for the majority of lymphocytes and other immune effector cells. The intestine is exposed to vast quantities of dietary and microbial antigens, and is the most common portal of entry for pathogens, some of which are potentially lethal. The development of normal immune function of the intestine is therefore vital for survival, and is dependent on appropriate antigen exposure and processing, and also an intact intestinal barrier. In early life innate mechanisms of defence are probably more important than active or adaptive mechanisms in responding to an infectious challenge, since the healthy neonate is immunologically naïve (has not seen antigen) and has not acquired immunological memory. During this period maternal colostrum and milk can significantly augment resistance to enteric infections. The mechanisms of enhancing disease resistance are thought to be passive, involving a direct supply of anti-microbial factors, and active, by promoting the development of specific immune function. A tolerance response to dietary and non-invasive antigens is generally induced in the gut. However, it must also be able to mount an adequate immune response to ensure clearance of foreign antigens. It is now recognized that regulation of tolerance and active immune responses is critical to health, and failure to regulate these responses can lead to recurrent infections, inflammatory diseases and allergies. The education of the immune system in early life is thought to be critical in minimizing the occurrence of these immune-based disorders. During this phase of development maternal milk provides signals to the immune system that generate appropriate response and memory. One factor that has been proposed to contribute to the increase in the incidence of immune-based disorders, e.g. atopic diseases in Western countries, is thought to be the increased prevalence of formula-feeding.

### Intestine: Immunity: Neonate: Colostrum

#### Organs and cell types involved in mediating immune responses

The immune system consists of organs and several cell types that recognize foreign antigens. The primary immunological organs consist of the bone marrow and the thymus, and the secondary organs include spleen, mesenteric lymph nodes and Peyer's patches. The immune cells can be grouped into two major categories, lymphocytes and phagocytes; the latter group includes monocytes, macrophages and neutrophils. The lymphocytes are exclusively involved in

the specific immune recognition of foreign antigens, whereas phagocytes function in the production of innate immune responses. The lymphoid progenitor cell has the capacity to differentiate into T-cells (T-helper (Th) 1 and 2) or B-cells, and this is largely determined by the micro-environment to which they migrate. T-cells develop from precursors in the thymus, whereas mammalian B-cells differentiate in the fetal liver and postnatal bone marrow. The major function of T-cells is in antigen recognition and clearance, and in coordinating the response of B-cells. B-cells produce antibody, and in the gut this is mainly an

**Abbreviations:** CD, cluster of differentiation; GALT, gut-associated lymphoid tissue; Ig, immunoglobulin; M-cell, microfold cell; TGF $\beta$ , transforming growth factor  $\beta$ ; Th, T-helper

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immunoglobulin (Ig) A isotype. Phagocytic cells are derived from myeloid progenitor cells, and function to remove particulate antigen by phagocytosis. Macrophages, which are mononuclear phagocytes, also have an important role in antigen presentation to T-cells.

### Structural development of the immune system in neonates

At birth the structural development of the immune system is extensive. In the same way as in the gut epithelium, the extent of maturation of the gut-associated lymphoid tissue (GALT) can be correlated with gestational length. The primary lymphoid organs, thymus and bone marrow, are generally well-formed and contain progenitor lymphomyeloid cells from very early in development. Lymphopoiesis is initiated early in gestation and is very advanced at birth. A communicating network of lymphatic vessels is present during early development, creating a functional circulatory system which enables rapid dissemination of lymphomyeloid precursor cells (Husband & Gleeson, 1996). This system permits seeding of precursor cells from progenitor sites to key inductive sites such as Peyer's patches and mesenteric lymph nodes, and ensures a parallel development of the primary and secondary lymphoid organs. T-cells, expressing T-cell receptor, and cluster of differentiation (CD) CD4<sup>+</sup> (Th) and CD8<sup>+</sup> (cytotoxic or suppressor) phenotype can be detected in the Peyer's patch germinal centres and lamina propria of the intestinal epithelium at 18 weeks of gestation (Insoft *et al.* 1996). Intraepithelial lymphocytes appear at 11 weeks of gestation, and by early postnatal life are almost entirely CD8<sup>+</sup> phenotype (Insoft *et al.* 1996). B-cells expressing CD5 surface antigen develop in the fetal liver and postnatal bone marrow, and are also associated with the epithelium early in gestation (Insoft *et al.* 1996). During late fetal life and early extrauterine life, B-cells undergo gene rearrangements that are independent of antigen and that result in a repertoire of approximately 10<sup>4</sup> specificities (Zanetti, 1992). Cells mediating the innate immune response, such as macrophages and neutrophils, are present early in gestation. Infiltration of these cells into the intestine requires an antigenic stimulus occurring after birth.

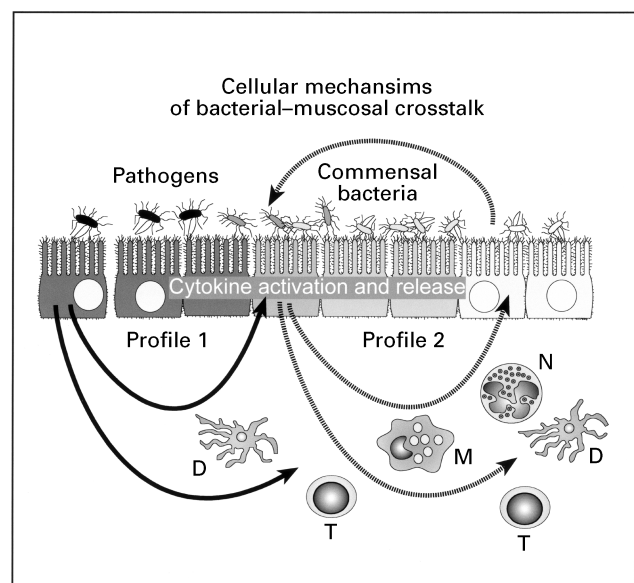
### Antigen exposure and immune development

Very little antigen exposure occurs *in utero*. Hence at birth, the immune system of a healthy neonate is naïve from an immunological standpoint. During early postnatal life exposure to antigen is a prerequisite for promoting the expansion of immune organs. In fact, rather as in the nervous system, the development of the immune system is based on experience, and the response to antigen primes and establishes the functional immune response early in ontogeny (Zanetti, 1992). Bacterial antigens play a very significant role in the proliferation and development of the GALT (Helgeland *et al.* 1996). This feature has been highlighted by investigations on gnotobiotics. It is noteworthy that germ-free animals, which are exposed to dietary antigens but not bacterial antigens, possess only a rudimentary immune system. The significance of the

microbial flora in relation to host immunity is further highlighted by the fact that the decreased exposure to microbes in Western society has been correlated with an increased incidence of atopic and autoimmune diseases in adults (Rook & Stanford, 1998).

The mechanisms by which microbes influence the phenotype and function of lymphoid cells associated with the GALT are largely unknown, but are likely to involve complex events that are probably triggered following the 'normal' route of antigen uptake and processing. Commensal bacteria and pathogens can also directly influence intestinal cytokine profiles (Fig. 1; Wilson *et al.* 1998; D Kelly, unpublished results) and these signalling molecules can have very dramatic effects on immune variables such as the polarization of the immune response and Th-cell subset development (Delespesse *et al.* 1998). Neonates tend to display preferential Th2 polarization of T-cells (Siegrist *et al.* 1998). Th1 and Th2 responses and their balance appear to be critical in mounting the strong cytotoxic responses required for pathogen clearance. The Th1–Th2 balance is, in turn, thought to be influenced by microbial exposure (Rook & Stanford, 1998), and perhaps suggests that there is an optimum flora in early life that can promote a healthy intestine and optimize its immune function.

In the neonate it is generally accepted that the generation of appropriate immune responses and the development of immune regulatory networks are dependent on the development of a normal intestinal flora and the exposure to dietary antigen (Brandtzaeg, 1996). Furthermore, the immunological outcome following exposure to antigen is determined by a number of variables, including genetic background and the nature, timing and dose of administered antigen (Strobel & Mowat, 1998). Clearly, the process of



**Fig. 1.** Pathogenic and commensal bacteria can influence intestinal barrier function (autocrine) and the activation of lymphoid cell populations (paracrine) via differential regulation of epithelial cytokine synthesis. D, dendritic cells; T, T-cells; M, macrophages; N, neutrophils.

introducing antigen in early life is important. Yet, the feeding regimens adopted from birth to weaning are based mainly on the perceived nutritional requirements of the neonate, and have not been established with an understanding of the immunological consequences associated with this process.

### Mechanisms of immune response

The primary function of the immune system is to eliminate infectious agents and to minimize the damage they cause. However, the immune system also has to process harmless antigens, and has evolved intricate mechanisms to discriminate between antigens with the potential to cause damage and those without. Failure to regulate intestinal immune responses appropriately can lead to disease such as food-related allergy, autoimmunity and inflammatory bowel disorders. Dietary antigens and bacterial antigens from the indigenous microflora trigger tolerance induction in healthy individuals, whereas pathogens induce strong activation of immune defence mechanisms required for pathogen clearance.

The immune defence relies on the two 'arms' of the immune system, the innate and the acquired. The innate immune system is believed to have evolved before the acquired or adaptive immune system, as it is found in all multicellular organisms, whereas the adaptive system is found only in vertebrates (Medzhitov & Janeway, 1997; Dyrinda & Ratcliffe, 1998). The major function of the innate immune response is as a first line of defence to effectively limit the spread of an infectious challenge. The major immune cell types involved in mediating innate responses are macrophages, neutrophils and natural killer cells. These cells are capable of discriminating self from non-self, and recognize molecular arrays or patterns that seem to be shared among groups of pathogens. Molecules such as lipopolysaccharides or teichoic acids provide the recognition base for gram-negative and gram-positive bacteria respectively, whereas double-stranded RNA is involved in virus recognition, and mannans provide the structural signature involved in yeast recognition (Medzhitov & Janeway, 1997). These structures are recognized by pattern-recognition receptors expressed on activated macrophages, and induce mechanisms of kill including phagocytosis and opsonization. Receptors thought to be important in regulating the innate host response include C-type lectins such as the collectins (Hansen & Holmskov, 1998), toll and toll-like receptors (Brightbill *et al.* 1999) and CD14 which is involved in the recognition of lipopolysaccharides. For many years the innate immune system has been considered as quite distinct from the adaptive system. However, recent studies have shown that their functions are integrated such that the innate system operates to initiate and regulate the adaptive response (Jullien *et al.* 1997).

The adaptive immune responses are initiated following antigen uptake and presentation to T- and B-cells. The major route of antigen uptake has long been considered to be via microfold cells (M-cells) which are located on organized lymphoid structures referred to as Peyer's Patches (Fig. 2). Antigens are transported into the GALT and are presented to

T- and B-cells by antigen-presenting cells, including dendritic cells and macrophages. This process involves antigen recognition and has inherent fine molecular specificity. Essential to the activation of T-cells is antigen presentation in combination with major histocompatibility complex, recognition involving the T-cell receptor, and a co-stimulation signal mediated through ligation of molecules such as B7 expressed on antigen-presenting cells or CD28 on T-cells (June *et al.* 1994). Co-stimulation is very important in T-cell activation, and absence of this signal leads to a partial T-cell response or tolerance induction. Following antigen priming, T and B lymphocytes migrate through the lymph and reach the peripheral blood for migration to mucosal effector sites. In this way, protection against environmental pathogens and allergens is transferred to mucosal surfaces in the entire intestine, via the secretion of specific secretory IgA from B lymphocytes (Kraehenbuhl & Neutra, 1992). This non-inflammatory mechanism of host protection inhibits colonization and invasion by pathogens by immune exclusion (Tomasi, 1983). As a result of migration of activated T-cells and B-cells to the mammary gland, the maternal experience of environmental antigens is conveyed to the sucking neonate (Fig. 2).

### Innate v. adaptive immunity in neonates

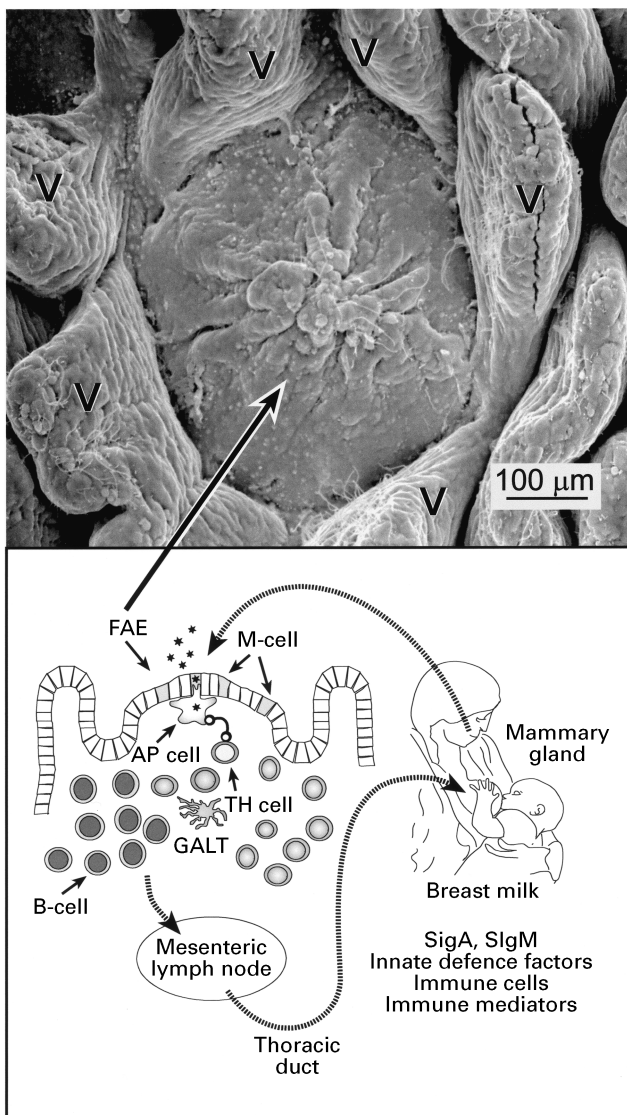
In the healthy adult resistance to infection relies on a balance between the innate and adaptive (antigen driven) immunity. However, in the neonate the adaptive arm of the immune system has not fully developed, and for example T-cell responses can be functionally limited because the cells are of naïve phenotype, have a lower level expression of co-stimulatory molecules such as CD40L and may also have greater threshold requirements for activation (Kovarik & Siegrist, 1998). Inadequacies in T-cell function will dramatically influence the efficiency of cell-mediated immunity. Other quantitative and qualitative differences in lymphomyeloid populations exist between adults and neonates (see next Section) and are likely to contribute to differences in immune competence (Bona & Bot, 1997).

Hence, during the neonatal period cells of the innate immune system, predominantly macrophages, neutrophils and natural killer cells, are largely responsible for the clearance of foreign antigen. In neonates, although the cells involved in triggering innate immunity are functional, they are present in lower numbers, are less chemotactic and have lower enzyme activity than their adult counterparts (Kovarik & Siegrist, 1998). Neonatal cells also have a limited ability to activate the specific adaptive arm of the immune system (Fairhurst *et al.* 1998).

### Functional limitations of protective immunity in neonates

As discussed earlier, the cellular apparatus of the immune system is in place, and mucosal and systemic antibody responses can be detected early in life. However, a functional deficit in immune function (both innate and adaptive) persists for some time after birth, leaving the neonate susceptible to bacterial and viral attack. Aspects of





**Fig. 2.** Antigen sampling in the maternal intestine occurs through microfold cells (M-cells) present in the follicle-associated epithelium (FAE) in the Peyer's patch regions. Activated lymphocytes leave the maternal intestine via lymphatic vessels and enter the maternal blood for distribution to distal effector sites, including the mammary gland. The maternal experience of antigen is then conveyed to the sucking infant. Scanning electron micrograph depicts rat Peyer's patch dome surrounded by intestinal villi (V). AP cell, antigen-presenting cell; Th cell, T-helper cell; GALT, gut-associated lymphoid tissue; SlgA, SlgM, secretory immunoglobulins A and M.

the immune function which account for the immature responses to antigen challenge include enhanced intestinal permeability and underdeveloped T- or B-cell function (Insoft *et al.* 1996; Brandtzaeg, 1998). In the neonate it has been proposed that a major limitation to mounting appropriate immune responses relates to deficiencies in antigen presentation, and in particular the function of antigen presenting cells (Ridge *et al.* 1996; Jullien *et al.* 1997; Medzhitov & Janeway, 1997). The neonate is known to be deficient in antibodies to cell-wall polysaccharides of encapsulated pathogens (Fairhurst *et al.* 1998), and this

deficiency is thought to contribute significantly to the susceptibility to bacterial infections during the neonatal period. Specifically, deficiencies in the CD1 system of antigen recognition and presentation are thought to limit the production of anti-polysaccharide antibodies (Fairhurst *et al.* 1998). The CD1 system is recognized as a third method of antigen presentation in addition to major histocompatibility complex I and II, and is thought to be important in non-peptide antigen presentation and to provide a link between the innate immune system and the adaptive response (Jullien *et al.* 1997). CD1 molecules have been found on thymocytes, professional antigen presenting cells, including dendritic cells (Jullien *et al.* 1997), B-cells in the intestinal and colonic lamina propria, and on intestinal epithelial cells (Fairhurst *et al.* 1998; Campbell *et al.* 1999). Recent data have shown that CD1-gp180 expressed on intestinal epithelial cells can recognize T-cell receptor-CD8 co-receptor and activate CD8 T-cells in the intestine (Campbell *et al.* 1999). It is, tempting, therefore to speculate that developmental deficiencies in the expression of CD molecules or other surface proteins involved in cell activation, e.g. co-stimulatory molecules, may represent a limitation in the presentation of microbial non-peptide antigens to neonatal T-cells. This limitation may explain why the neonate fails to mount strong immune responses and, given an appropriate level of pathogen exposure, why its mucosal surfaces may be colonized by harmful micro-organisms.

#### Non-immune mechanisms contributing to the disease susceptibility of neonates

Factors other than immaturity of the immune system can contribute to disease susceptibility. For example, within the intestine, epithelial cells are known to possess a diversity of cell-surface carbohydrate structures that project into the intestinal lumen. These structures are developmentally regulated (Taatjes & Roth, 1990) and provide specific binding sites for intestinal bacteria. The expression of these glycans can therefore have dramatic effects on the colonizing microflora. A recent definitive study investigating the susceptibility of young pigs to *Escherichia coli* K88 infections found that the disease-susceptible pigs possessed a specific galactosylated receptor structure that was completely absent from resistant animals (Jeyasingham *et al.* 1999). Similar observations have been reported on the expression of the K99 *E. coli* receptor, a sialylated structure that renders young pigs and calves highly susceptible to infections (Seignole *et al.* 1991). Clearly, heightened expression of bacterial receptors at certain times during postnatal development creates windows of susceptibility during which opportunistic pathogens are able to colonize mucosal surfaces. With high-level receptor expression and an immature immune system, the relatively high susceptibility to intestinal bacterial and viral diseases in early life is not surprising.

A further feature of the intestine of neonates that is likely to be important to immune development and disease susceptibility relates to the barrier function of the intestinal epithelium (Insoft *et al.* 1996). It is generally recognized that the intestine of neonates is more permeable than that of



the adult, and hence more at risk with regard to transfer and uptake of potentially harmful lumen antigens, including pathogens. Important structural components of the intestinal barrier are the junctional proteins, such as occludin and the claudins. These junctional components play an important role in the adhesion and sealing between epithelial cells, and their expression is likely to be developmentally regulated and possibly influenced by nutrition. Conceivably, the presence of biologically-active factors in maternal secretions may promote the enhanced expression and function of junctional structures, and thereby contribute to the general protective nature of mammalian milk.

### Early nutrition and the developing immune system

It has been long recognized that infants who are breast-fed succumb to fewer infections compared with those who are formula-fed. In both developed and developing countries there appears to be consistent evidence for a protective effect of breast-feeding in the first 4–6 months (Golding *et al.* 1997). The protective benefit of breast-feeding was previously thought to be associated with the fact that breast milk is sterile, whereas formula-feeding carries a significant risk of bacterial contamination during preparation. However, it is now accepted that maternal colostrum and milk, in addition to providing the newborn with nutrition, also confer immunological advantages and protect against gastrointestinal and respiratory diseases. In addition to passive protection, maternal milk may have the capacity to directly stimulate immune function. Although the precise mechanisms by which maternal milk confers protection against disease are poorly defined, it is likely that the benefits involve altered intestinal physiology, microbiology and immunology. The protective mechanisms conferred by maternal colostrum and milk in the suckling infant can be grouped into four main categories comprising maturational, immuno-modulatory, anti-inflammatory and anti-microbial effects.

### Maternal milk and maturation of the intestine

Colostrum and milk feeding have been shown to promote the maturation of the developing intestinal epithelium (Kelly & King, 1991; Burrin *et al.* 1992; Kelly *et al.* 1993; Wang & Xu, 1996). Lactase expression (specific and total) was found to decline significantly in colostrum-fed pigs when compared with colostrum-deprived animals (Kelly *et al.* 1991). The levels of membrane sialylation and fucosylation on epithelial cells indicated that cells from colostrum-fed animals were phenotypically more mature than those of formula-fed animals (King & Kelly, 1991; Kelly *et al.* 1993). Differences in glycosylation patterns over Peyer's Patch epithelium were also observed. As discussed earlier, carbohydrate moieties expressed on the surfaces of epithelial cells provide important binding sites for bacteria and viruses. It may be anticipated that variations in glycosylation complexation can contribute to qualitative and quantitative differences in the adherent microflora of breast-fed and formula-fed infants. Furthermore, the adherent flora in colostrum-fed animals may be qualitatively

different over Peyer's Patch epithelium, and hence both the nature and quantity of microbial antigens sampled by M-cells may also differ. The possibility that such differences influence the priming of the immune system is considerable, since the nature and dose of antigen can have a dramatic effect on immunological outcome (Strobel & Mowat, 1998). Equally, the ability of different bacterial species to differentially influence the cytokine milieu within the intestine (Wilson *et al.* 1998) can also dramatically influence immunological events and Th subset development (Delespesse *et al.* 1998).

### Maternal milk and immune modulation

The immuno-modulatory effects of maternal milk have been mainly investigated using antibody (humoral) responses to vaccines as probes of immunity. The results from these studies are controversial. However, a well-executed randomized trial was recently reported, showing that breast-fed infants exhibit enhanced specific antibody titre to some, but not all, vaccines (Pickering *et al.* 1998). Very little work has been carried out on the cell-mediated immune response in breast-fed *v.* bottle-fed infants. However, some recent studies have shown that immunophenotypic differences in lymphocyte populations occur following exposure to maternal milk. These differences include a decrease in CD4<sup>+</sup>:CD8<sup>+</sup> cells, an increase in interferon- $\gamma$  levels and a greater number of natural killer cells (Pabst *et al.* 1997; Hawkes *et al.* 1999), and are consistent with age-related changes, suggesting that maternal milk can influence the maturation of the neonatal immune system (Hawkes *et al.* 1999). The mechanism whereby maternal milk may induce these effects is not known.

The previous examples highlight studies showing that maternal feeding can augment the development and function of the neonatal immune system. However, there is also a considerable body of literature describing immuno-suppressive effects of breast-feeding and maternal antibodies on the development of active immune responses (Rennels, 1996; Vesikari & Joensuu, 1996; Hodgins *et al.* 1999). The immuno-suppressive effect may be due to the possibility that secretory IgA reduces the uptake of dietary and bacterial antigens, and therefore reduces their contact with the GALT (Brandtzaeg, 1998). Breast milk also contains immuno-suppressive factors such as interleukin 10 and transforming growth factor  $\beta$  (TGF $\beta$ ) (Letterio *et al.* 1994; Garofalo *et al.* 1995). The immuno-suppressive properties of breast milk may facilitate tolerance induction to harmless food antigens and antigens associated with commensal bacteria (Brandtzaeg, 1998), and may explain why breast-fed infants have a lower incidence of food-related allergies (Saarinen & Kajosaari, 1995). However, the possibility that passively-acquired antibodies may influence the magnitude or nature of the immune response and alter the Th1–Th2 balance (Hodgins *et al.* 1999) is important in the context of vaccination of neonates. For young infants with high levels of passively-acquired maternal antibodies it may be necessary to adopt strategies to improve vaccine efficacy, including manipulation of the vaccine titre, timing and frequency of dose, addition of adjuvants and micro-encapsulation to ensure protection (Hodgins *et al.* 1999).

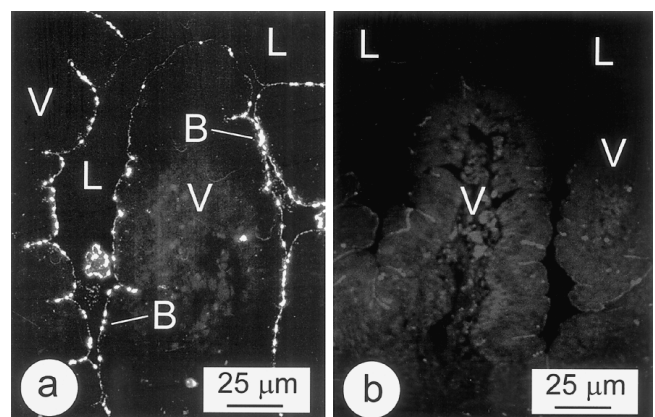
From a mechanistic point of view, it has been postulated that maternal lymphocytes in milk fulfill an important role in modulating neonatal immune responses. The majority of lymphocytes in milk are T-cells (83%; Goldman & Goldblum, 1997). Maternal T-cells are present in a very activated state and express the surface antigen CD45RO<sup>+</sup>, associated with immunological memory (Bertotto *et al.* 1990). The presence of lymphocytes with immunological memory is thought to be due to the homing of these cells from the maternal intestine to the mammary gland. There is also experimental evidence confirming that milk lymphocytes can attach and traverse the neonatal intestine, and can remain locally within the intestine or migrate to enter the circulation (Goldman & Goldblum, 1997; Xanthou, 1997). Milk lymphocytes express very high levels of ligands such as CD40L (Bertotto *et al.* 1996). These cells are thought to compensate for the immature function of neonatal T-cells by providing potent activation signals leading to strong active immune responses that enhance the defence mechanisms of the neonate (Bertotto *et al.* 1996; Xanthou, 1997). It has also been proposed that milk-borne cytokines are important regulators of immune responses. For example, human colostrum has been shown to stimulate the release of cytokines from peripheral blood mononuclear cells, thus altering the cytokine milieu or background against which immunological events are instigated (Bessler *et al.* 1996; Delespesse *et al.* 1998).

#### Maternal milk and anti-inflammatory function

It is interesting that the defence factors in human milk, such as secretory IgA, function without causing inflammation. Some maternal milk constituents have been reported to have anti-inflammatory activity. Lactoferrin and fragments of lactoferrin have been shown to inhibit the endotoxin-induced interleukin 6 release from human monocytic cells (Mattsby-Baltzer *et al.* 1996). The cytokines interleukin 10 and TGF $\beta$  have been documented in maternal milk, and are also recognized to have immuno-suppressive and anti-inflammatory activity (Letterio *et al.* 1994; Garofalo *et al.* 1995). Maternal TGF $\beta$  has been shown to be important to the survival of TGF $\beta$ -null mice, and functions by reducing the diffuse and lethal inflammation caused by gene disruption (Letterio *et al.* 1994). TGF $\beta$  was found to be present in lymphocytes and macrophages isolated from mouse milk, suggesting that these cells have the biosynthetic capacity for this cytokine. Further studies have shown that TGF $\beta$  is rapidly taken up by neonatal tissues, including the intestine, which suggest that this protein may have an important role in postnatal development (Letterio *et al.* 1994) and may play a crucial role in regulating inflammatory immune responses generated in vulnerable neonatal tissues. Interleukin 10 also inhibits pro-inflammatory cytokine release, limits Th1 reactions and interacts synergistically with TGF $\beta$  to augment IgA synthesis (Garofalo *et al.* 1995). Hence, the function of interleukin 10 and TGF $\beta$  in human milk is possibly to enhance non-inflammatory defence mechanisms at mucosal surfaces and to suppress potentially damaging inflammatory immune reactions.

#### Maternal milk and anti-microbial function

The protection afforded by maternal milk has been largely attributed to the presence of secretory IgA (Brandtzaeg, 1998). Breast milk has a higher level of IgA than serum. IgA prevents attachment and invasion of pathogens in the intestine by competitively binding and neutralizing bacterial antigens (Schroten *et al.* 1998). However, milk also contains a large number of other components with anti-microbial activity, including complex carbohydrates, glycoproteins, glycolipids, glycosaminoglycans, mucins and oligosaccharides (Newburg, 1999). The oligosaccharides comprise the third most abundant constituent in milk and contain a myriad of structures. Those oligosaccharides with homology to cell surface pathogen receptors may inhibit pathogen interactions with host mucosal tissues and thereby protect against infection. A large number of milk constituents reportedly interfere with pathogen binding: lactadherin is a milk glycoprotein that inhibits rotaviral infection (Newburg *et al.* 1998), while IgA and mucin prevent the attachment of S-fimbriated *E. coli* to epithelial cells due to the presence of sialic acid residues (Schroten *et al.* 1998). The anti-microbial activity of IgA can be attributed to both the antigen-binding fragment-mediated neutralization of viruses and bacteria, and also to the presence of specific glycans, which present as receptor sites for pathogens. Receptor analogues for K88 *E. coli* have also been identified in bovine milk, and include two glycoproteins of molecular weight 18 and 25 kDa shown *ex vivo* and *in vitro* to prevent K88 fimbrial binding to weaned pig intestine (Fig. 3; MD Jeyasingham, P Butty, TP King, R Begbie and D Kelly, unpublished results). Glycans found on bovine lactoferrin also function as receptors for type 1 fimbrial lectin of *E. coli* (Teraguchi *et al.* 1996). Maternal milk clearly contains a diverse range of components that display carbohydrate structures that function as receptor sites for many common environmental pathogens. These components must contribute significantly to the protection



**Fig. 3.** Bovine milk glycoprotein inhibitor of K88 *Escherichia coli*. (a) Labelling of intestinal brush border with K88 fimbriae detected with fluorescent-conjugated anti-K88 antibody on resin sections of villi from a K88 susceptible pig. (b) The same binding assay was undertaken in the presence of a bovine glycoprotein inhibitor that functions as a receptor analogue and prevents K88 *E. coli* attachment to villus surfaces (MD Jeyasingham, P Butty, TP King, R Begbie and D Kelly, unpublished results). V, villus; B, bacteria; L, lumen.

of neonatal mucosal tissues against bacterial and viral attack, and are very likely to be major players in the reduced incidence of gastroenteritis associated with breast-feeding.

Bifidus factors and other prebiotics are thought to enhance beneficial bacterial colonization of the gut (Kunz *et al.* 1999). Maternal milk is known to promote the establishment of lactic acid bacteria and bifidobacteria. These groups of organisms are thought to reduce the pathogenic potential of bacteria in the gut by altering pH, by secreting specific antibiotic-like substances and/or by reducing the invasive potential of pathogens (Silva *et al.* 1999).

### Early nutrition and immune-based disorders

Atopic, autoimmune and inflammatory diseases are all immune-based disorders that are generally becoming an increasing problem. Exclusive breast-feeding has been associated with a reduction in the incidence of these disorders (Chandra & Hamed, 1991; Saarinen & Kajosaari, 1995), largely because the exposure to 'foreign' dietary and environmental antigens is avoided or reduced during the period when the immune system is physiologically immature. There are discrepancies in the literature concerning the relationship between breast-feeding and the incidence of atopic disease. For example, Saarinen & Kajosaari (1995) presented data for 150 children and concluded from their work that breast-feeding is prophylactic against atopic diseases including eczema, food allergy and respiratory allergy. However, other workers have reviewed the literature and have expressed more cynical viewpoints with respect to evidence supporting these claims (Wold & Alderberth, 1998). A recent paper on the incidence of autoimmune diseases, in particular type 1 diabetes, provides evidence linking this disease to the early introduction of cow's milk (Kolb & Pozzilli, 1999). The authors also suggest that constituents in cow's milk can interact with cells in the immune system and negatively influence tolerance mechanisms. If substantiated, this interaction may be relevant to both autoimmune and atopic diseases.

It could be postulated that during early postnatal life, the prime function of immune reactivity is to acquire immunological tolerance to normal dietary and environmental antigens. The presence of pathogens may disturb immune tolerance, as a result of 'bystander' effects. Hence, non-immune control of pathogens and other foreign antigens by anti-microbial substances in milk protects the sucking infant, but importantly also minimizes the demand for active immune responses until an appropriately responsive immune system is established.

### Summary and conclusion

The immune system is functionally immature at birth. This immaturity leaves the neonate susceptible to viral and bacterial attack. Maternal milk contains a diverse complement of factors that can promote the maturation of function of neonatal tissues, and also protect them by passive mechanisms. The evidence for passive protection employing the panoply of anti-microbial factors in milk is

considerable. The function of these anti-microbial factors is obviously to suppress pathogenic challenge, but importantly may also prevent aggressive active immune responses at a time when the developing immune system is undergoing 'fine-tuning'. The effect of maternal nutrition on the functional development of the infant's immune system is controversial. The evidence that breast milk exerts immunosuppressive effects but can also promote active immunity is difficult to reconcile. However, recent data appear to suggest that the suppressive effects are relatively short-lived, and that the breast-fed infant exhibits augmented immune responsiveness within 8 months from birth (Brandtzaeg, 1998). The view that the 'development or education' of the immune system is critically-regulated during the breast-feeding period, and that this regulation is important to both short-term and long-term health, particularly in the context of immune-based disorders, requires further investigation. Understanding the relationship between breast-feeding and immune development at a mechanistic level, with the help of the extensive progress made by immunologists, will undoubtedly clarify the benefits of maternal feeding.

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