Development of digestive and immunological function in neonates: role of early nutrition

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Abstract

The developing intestine undergoes morphogenesis and cytodifferentiation, events that are exquisitely regulated and highly organised, both spatially and temporally. The outermost epithelial layer of the intestine, the site of nutrient digestion and absorption, is organised into two morphological and functionally distinct compartments. Within the crypt, stem cells undergo several cycles of cell division and migrate onto the villus where they exhibit changes in phenotype and functional characteristics. Enzyme, transporter and glycosylation patterns change dramatically during the transition from birth to adulthood. These transitions permit dietary change and adaptation, and are also important determinants of disease susceptibility. In addition to its role in nutrient uptake, the intestine represents a key innate component of intestinal defence by providing a physical barrier to the external environment. Maintenance of tight junctions and the ability to secrete mucin are critical components of its protective function. Innate immunity is particularly important to the neonate, as the active or specific arm of the immune system is immature. In early life, the presence and processing of luminal antigen, particularly bacterial antigen, drives the expansion of intestinal epithelial and lymphoid tissues. Early nutrition is also recognised to play a very significant role in modulating intestinal differentiation and function and in regulating immune responses to environmental antigens. © 2000 Published by Elsevier Science B.V.

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1. Introduction

The intestinal epithelium represents the major interface between the host and the environment. It plays a crucial role in nutrient transport and in protection, by limiting the uptake and translocation of harmful agents. The expression of biologically active enzymes and transporters, that degrade and transport nutrients from the intestinal lumen across the epithelium, is essential to the digestive function of the intestine. Many of these membrane-bound glycoproteins are developmentally regulated. A change in the expression of these enzymes and transporters provides the cellular basis for developmental changes in digestive capability and capacity. Dramatic changes in glycosylation patterns also occur during development and are thought to be important to intestinal health. Many of the carbohydrate moieties displayed on gut surfaces are im-
portant determinants of bacterial colonisation and uptake by epithelial cells, including M cells. The barrier function of the intestinal epithelium is an important feature of the intestine at all stages of life, and is reinforced by the formation of tight junctions, which dramatically reduce the permeability of the intestine. The mucin gel coating the intestine provides a further protective feature that can trap noxious agents and thus prevent their access to the intestinal epithelium. Carbohydrate moieties, which are expressed in abundance in mucin, are thought to restrict the movement of harmful agents within the mucous gel.

A major function of the intestinal epithelium is to transport and present dietary and bacterial antigens to the immune system. For the developing neonate, intestinal transport of antigen is vital to drive the expansion and maturation of lymphoid organs. However, the neonate is immunologically naive and is very susceptible to infection and damage from harmful antigens. Early nutrition plays an important role in protecting the developing intestine from harmful agents and in modulating immune responses following antigenic challenge. Maternal milk provides a diverse range of substances that are developmentally delayed in the neonate and are thought to play a critical role in gastrointestinal defence. Bioactive molecules such as immunoglobulin A (IgA) and lactoferrin are present in abundance and are potent anti-microbial agents. Maternal milk also contains substances, including growth factors and cytokines, that promote the maturation of the intestine and the associated immune system. The immuno-modulatory property of maternal colostrum and milk is now attracting considerable scientific and commercial interest. Certain constituents of maternal milk, with immuno-modulatory potential, are capable of promoting immune function in early life, the benefits of which may persist throughout adult life. Identification of these constituents would permit supplementation of animal and infant milk formulations to improve their nutritional and health promoting properties.

This review discusses some of the major features of digestive and immune development in neonates and highlights the role of early nutrition in promoting intestinal function and health.

2. Development of intestinal epithelium and digestive function

Transitions in the morphogenic, phenotypic and functional characteristics of the intestinal epithelium occur in a very organised and predictable fashion during pre- and postnatal development. The intrinsic genetic programming of the intestine drives these developmental transitions. However, functional development can be altered by intrinsic factors, including neural and hormonal stimuli, but also by cell–cell and cell–matrix crosstalk or communication. In addition, diet and microbes influence both the proliferation and differentiation state of the epithelium and have a very significant impact on the developing intestine.

Extensive structural and functional development of the intestine occurs in utero. The degree of maturity at birth generally reflects the length of the gestational period. For example, in the human foetus at 20 weeks gestation, intestinal morphology and functional characteristics generally resemble those of the newborn (Lebenthal, 1989). Intestinal development in the pig, like the human, is also initiated early in foetal development. For example, at 40 days of gestation, intestinal morphogenesis has progressed to the point where recognisable villi are present and mRNA for enzymes and cytoskeletal proteins are readily detectable (Perozzi et al., 1993). The mammalian intestine undergoes two main phases of development during the neonatal period. The first phase involves the preparation for extra-uterine life when maternal colostrum and milk will provide the sole nutrient source. The second phase of intestinal development is associated with a shift in the digestive capability of the epithelium from one which digests and absorbs a milk diet to one which efficiently utilises complex solid feeds. With both these phases it is recognised that extensive changes occur in the gross architecture, ultrastructure, growth and differentiation of the intestine.

During the immediate postnatal period a dramatic increase in the diameter, total weight and surface area of the intestine occurs (Zhang et al., 1997). Total intestinal and mucosal weight has been reported to increase by 58% and 80%, respectively during the first 6 h of suckling and mucosal DNA
content to be 4.6-fold higher at 24 h after birth (Zhang et al., 1997). This increase in growth is driven by accelerated cell proliferation in the crypt stem cell compartment of the intestine. Furthermore, it has been proposed that a major component of the growth response of the intestine in early life is directed towards cryptogenesis and villus remodelling (Goodlad and Wright, 1990). In addition to extensive epithelial cell proliferation, dramatic changes in enzymes, receptors and transport systems occur. Many of the intestinal-specific genes, including those of enzymes, show distinct temporal patterns of expression. Lactase, the principal carbohydrate of the neonatal mammalian intestine and responsible for the digestion of lactose in milk, is very high at birth and declines with age and following the weaning process (Kelly et al., 1991a). A reciprocal pattern of expression occurs for enzymes such as sucrase and maltase. At birth, the levels of active enzyme are low and increase as the neonate matures or is weaned. The expression of these enzymes can be readily induced by presentation of specific substrates (Kelly et al., 1991b; Zhang et al., 1997). In the intestine, oligosaccharide moieties are found associated with enzyme and non-enzyme glycoconjugates. Many of these oligosaccharides provide a wide range of binding sites for luminal and circulating biologically-active ligands such as growth factors, hormones, bacteria and toxins (Kelly et al., 1992; Stewart et al., 1993). Age-related changes in intestinal glycosylation in neonates have been extensively reported (Taatjes and Roth, 1990; Kelly and King, 1991; King and Kelly, 1991). These changes play an important role in modifying the properties of intestinal receptors for dietary constituents as well commensal and pathogenic bacterial receptors and their evolution during development. Using lectins and carbohydrate-specific monoclonal antibodies for cytochemical detection of specific carbohydrate structures, a reciprocal relationship between membrane and goblet cell α 2,6 sialylation and α 1,2 fucosylation has been reported in the pig intestine during development (King and Kelly, 1991). These temporal glycosylation events are pre-programmed but are sensitive to dietary regime such as, colostrum and milk feeding and to weaning (Kelly and King, 1991; Kelly et al., 1993).

The carbohydrates expressed on the surface of gut cells are important determinants for bacterial colonisation as they facilitate cell recognition and bacterial attachment. Many of the pathogenic bacteria associated with the neonatal period have high affinity for carbohydrate moieties that are expressed in the intestine during this critical period. It is therefore likely that predisposition to certain bacterial and viral infections in the neonatal period are, in part, attributable to the presence of specific carbohydrate receptors. Recent studies with neonatal pigs have shown that the susceptibility to K88 Escherichia coli, a major pig pathogen, is related to the presence of a galactosylated receptor complex in the intestine of disease-susceptible animals, one that is absent from animals that display disease resistance (Jeyasingham et al., 1999).

3. Development of immune function in neonates

At birth the structural development of the immune system is complete. Analogous to the gut epithelium, the degree of maturation can be correlated with gestational length. The primary lymphoid organs, thymus and bone marrow, are generally well formed and contain progenitor lymphomyeloid cells. Lymphopoiesis is initiated early in gestation and is very advanced at birth. A communicating network of lymphatic vessels is also present during early development, creating a functional circulatory system which enables rapid dissemination of lymphomyeloid precursor cells. This permits seeding of precursor cells from progenitor sites to key inductive sites such as Peyer’s patches and mesenteric lymph nodes (MLNs).

The primary function of the immune system is to eliminate infectious agents and to minimise the damage they cause. Immune defence relies on the two arms of the immune system, the innate and the acquired. The innate immune system is believed to predate the acquired or adaptive immune system as it is found in all multicellular organisms, whereas the adaptive is found only in vertebrates (Medzhitov and Janeway, 1997; Dyrway and Ratcliffe, 1998). The major function of the innate immune response is 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 recognise molecular arrays or patterns, such as lipopolysaccharides or techoic acids, that seem to be shared among groups of pathogens. These structures are recognised by pattern recognition receptors expressed on activated macrophages that induce killing mechanisms including phagocytosis and opsonisation. For many years the innate immune system has been seen as a separate entity from the adaptive system. However, recent studies have shown that they have a more integrative function where the innate system functions 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 M cells which are located on organised lymphoid structures referred to as Peyer's Patches. Antigens are transported into the gut associated lymphoid tissue and are presented to T and B cells by antigen presenting cells (APCs), a process that involves antigen recognition and has inherent fine molecular specificity. Antigen-primed T and B lymphocytes migrate through the lymph and reach the peripheral blood, where they home to mucosal effector sites, including the mammary gland. In this way the maternal experience of environmental antigens is passed to the sucking neonate. The role of enterocytes and dendritic cells in antigen uptake and presentation to APCs (Kaiserlian and Etchart, 1999) is currently very topical and it is considered that immunological outcome may be determined as a result of the communication and crosstalk between these cell types.

Resistance to infection relies on a harmonious balance between the innate and adaptive (antigen-driven) immunity. However, in the neonate the adaptive arm of the immune system has not developed and, hence, during this period of vulnerability, cells of the innate immune system, predominantly macrophages, neutrophils and NK cells, clear large quantities of foreign antigen. Furthermore, although the neonatal cells are functional, they are present in lower numbers, are less chemotactic and have lower enzyme activity than their adult counterparts. Neonatal cells also have a limited ability to activate the specific adaptive arm of the immune system (Fairhurst et al., 1998).

3.1. Disease susceptibility of neonates

Although the cellular apparatus of the immune system is in place and mucosal and systemic antibody responses can be detected early in life, a functional deficit in immune function persists for some time leaving the neonate susceptible to bacterial and viral attack. Aspects of the immune function which account for the impaired responses to antigen challenge have been proposed and include enhanced intestinal permeability and immaturity in T and B cell function. However, in the neonate, a major limitation to mounting appropriate immune responses appears to relate to deficiencies in antigen presentation and, in particular, the function of APCs (Ridge et al., 1996; Jullien et al., 1997). The neonate is also very deficient in anti-polysaccharide antibodies to encapsulated pathogens (Fairhurst et al., 1998) and this has been attributed to deficiencies in the CD1 system of antigen recognition and presentation. The CD1 system is also thought to be an important antigen presentation pathway that links together the innate immune system and the adaptive response (Jullien et al., 1997). This may explain why the neonate fails to mount strong and appropriate immune responses. The neonate is therefore immunologically suboptimal and, given an appropriate level of pathogen exposure, the mucosal surfaces are readily colonised by harmful micro-organisms.

4. Early nutrition and the developing intestine and immune system

Maternal colostrum and milk not only provide the newborn with nutrition but also confer passive immunity and generally protect against gastrointestinal and respiratory diseases. In addition, maternal milk may have the capacity to directly stimulate immune function. Although the precise mechanisms whereby maternal milk confers protection are poorly defined, it is likely that they involve altered intestinal physiology, microbiology and immunology. The mode of action of factors in milk which confer
protection can be grouped into four main categories and comprise maturational, immuno-modulatory, anti-inflammatory and anti-microbial effects.

4.1. Maternal milk and maturation of the intestine

Colostrum and milk feeding have been shown to promote the maturation of the developing intestinal epithelium. Lactase expression (specific and total) was found to decline significantly in colostrum-fed animals when compared with colostrum-deprived animals. The levels of sialylation and fucosylation on epithelial cells indicated that intestinal cells from colostrum-fed animals were phenotypically mature (Kelly et al., 1993). Differences in glycosylation patterns over Peyer’s Patch epithelium were also observed. This suggests that the adherent flora in colostrum-fed animals may be qualitatively different over Peyer’s Patch epithelium and hence the antigens sampled may also differ. The possibility that such differences influence the priming of the immune system is considerable, since the nature and dose of antigen have a dramatic effect on the immunological outcome.

4.2. Maternal milk and immune modulation

The immuno-modulatory effects of maternal milk have been investigated using antibody responses to vaccines as indicators of immunity (Pickering et al., 1998). The results from these studies are very controversial. However, a well-executed randomised trial (Pickering et al., 1998) recently reported that breast-fed infants do exhibit enhanced specific antibody titre to some vaccines. Similarly, immuno-phenotypic differences in lymphocyte populations have been reported following exposure to maternal milk. These differences include a decrease in the ratio of CD4+ :CD8+ cells and a greater number of NK cells (Hawkes et al., 1999). This difference is consistent with age-related changes, suggesting that maternal milk induces maturation of the developing immune system. The mechanism whereby maternal milk induces these effects is largely unknown. Recent studies suggest that maternal lymphocytes in milk may fulfil an important role in modulating neonatal immune responses. Maternal lymphocytes are present in milk in a very activated state and express the surface antigen CD45RO, which is associated with immunological memory (Bertotto et al., 1990). They are also loaded with ligands such as CD40L (Bertotto et al., 1996). These cells are thought to be taken up by the neonate and to compensate for the immature function of neonatal cells by providing potent activation signals leading to strong active immune responses (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 (PBMCs), thus altering the cytokine milieu against which immunological decisions are made (Bessler et al., 1996). Among all the factors that are known to control T cell development and function, cytokines are considered to be the most important (Delespesse et al., 1998).

It is interesting that the defence factors in human milk function without causing inflammation and in fact a number of maternal milk constituents have been reported to have anti-inflammatory activity. Lactoferrin and fragments thereof has been shown to inhibit the endotoxin-induced interleukin 6 (IL6) release from human monocytic cells (Mattsby-Baltzer et al., 1996). The cytokine IL10 and TGFβ have been reported in maternal milk and are recognised to have immuno-suppressive and anti-inflammatory activities (Letterio et al., 1994; Garofalo et al., 1995). Maternal TGFβ has been shown to be important to the survival of TGFβ-null mice by reducing the diffuse and lethal inflammation caused by gene disruption (Letterio et al., 1994).

4.3. Maternal milk and anti-microbial function

The protection afforded by maternal milk has been largely attributed to the presence of secretory IgA. However, milk contains a large number of other components with anti-microbial activity including complex carbohydrates, glycoproteins, glycolipids, glycosaminoglycans, mucins and oligosaccharides. The oligosaccharides comprise the third most abundant constituent in milk and contain a myriad of structures. Those with homology to cell surface pathogen receptors may inhibit pathogen interactions with host mucosal tissues and therefore protect
against infection. A large number of constituents which interfere with pathogen binding have been reported in milk. Lactadherin is a milk glycoprotein that inhibits rotaviral infection (Newburg et al., 1998). 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 Fab-mediated neutralisation of viruses and bacteria, and 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 with molecular masses of 18 000 and 25 000. These have been shown to be efficacious in removing K88 fimbriae from the weaned pig intestine (unpublished observations). 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 are likely to contribute significantly to the protection of neonatal mucosal tissues.

A final comment on the presence of bifidus factors or prebiotics in maternal milk: these are thought to beneficially alter bacterial colonisation 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 by reducing the invasive potential of pathogens (Silva et al., 1999).

5. Conclusions

The intestine and immune system are functionally immature at birth. This immaturity leaves the neonate susceptible to viral and bacterial attack. Maternal milk appears to have a host of factors that can mature the function of neonatal tissues but also protect them by passive mechanisms. The effect of maternal nutrition of the functional development of the immune system is somewhat controversial. However, it is likely that well-conducted experiments that will address this question at a mechanistic level, will unravel further virtues of maternal feeding.

References


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