

Bioactive molecules in milk: homeostatic function in the intestinal mucosa

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Introduction.

Experimental evidence generated during the last years is helping to unravel a complex bidirectional interaction between the host and its intestinal microbiota. Several studies support the contention that there is a mutualistic or symbiotic relationship which leads to increased performance of both partners (1). Our own interest has been to characterise interactions between commensals and mucosal immune cells, including epithelial cells (IEC) (2).

During postnatal development, the intestinal microbiota represents a major environmental modulator of epithelial gene expression and indeed, of the mucosal immune system in general. Later in life it has been postulated to provide an energy source, maintain immune homeostasis and strengthen defense against pathogens (3, 4).

At the cellular level, host-commensal interactions imply host cell recognition of bacterial cells or their conserved molecular patterns and subsequent cell signalling. The classical view of infectology is that such recognition leads to microbial clearance from the body with some inflammatory damage of host tissues. Intestinal immune responses to the resident microflora do not result in either of these. Thus, successful regulation of processes involved in recognition of non-pathogenic bacteria and true pathogens is the foundation of intestinal health. Its failure is associated with inflammatory bowel damage or gut derived infections in early in life and specific clinical manifestations in adulthood.

The essential functional features of the immune system are to recognise and react to "non-self" or "danger signals" on the one hand but remain tolerant to its own cells on the other. This requires specific instruction of immunocompetent cells in the thymus and the peripheral tissues during development. To handle colonisation by the intestinal microbiota after birth, the immune system most probably requires a postnatal "instruction period" that depends on contact between immunocompetent cells in the mucosa and luminal antigens, bacterial cells and compounds. Other environmental signals, such as breast milk borne factors, may also play a role in the instructive processes. However, the mechanisms underlying the "decision" to eliminate or to tolerate an intruder are far from being totally understood.

It should also be borne in mind that in this bidirectional relationship, the construction of the complex microbial ecosystem of the gut is influenced by the host. The gut associated lymphoid tissue (GALT) in particular, plays an important role in shaping the microbiota (5). By sampling intestinal bacterial antigens, this compartment activates both T-cell dependent and independent IgA production which may subsequently define microbial diversity through suppressing outgrowth of specific subpopulations (2). It may also contribute to the maintenance of immunological tolerance to commensals (6). In either case, inflammatory tissue damage does not ensue.

The differential stimulation of the gut immune system involves a variety of bacteria which ranges from autochthonous to allochthonous organisms and pathogens.

The intestinal ecosystem interacts with a continuum of symbiotic autochthonous members of the microbiota, transitory allochthonous microbes which are capable of triggering infection and virulent pathogens (7, 8).

While the cellular response to pathogenic microorganisms has been extensively studied, the host response to normal components of the microbiota is considerably less characterised.

Nevertheless, several features of both commensal bacteria and pathogens are known to influence the host response. These include the organism's capacity to occupy and colonise an intestinal niche, its interaction with or invasion of specific cell types, as well as its capacity to induce cytopathic effects or to access distinct mucosal compartments.

Pathogenic gene activation of immune cells involves two 'sets' of genes: a cluster of genes that is activated by most human pathogens as well as a cluster that is defined by specific virulence traits of a given pathogen (9). It is likely that the former results from the recognition of the "non-self danger signal" by cellular receptors, such as the toll-like receptors (TLR), that are expressed on sentinel cells like dendritic and IEC at the mucosal surface (10). This recognition is part of the innate response which influences subsequent, adaptive immune responses.

Until recently, it was commonly believed that the host-commensal interaction was nothing more than "immunological ignorance" of the host. However, some studies now suggest that it involves receptor-mediated recognition of the bacteria or their products and subsequent signal transduction. Moreover, this recognition seems to depend, at least in part, on the same receptors that interact with pathogens (11). In general, most bacteria, pathogenic or not, promote pro-inflammatory gene activation in different cell types. However, in physiological conditions in the intestine, if a non-pathogen induces such a response, it is only transient (12). The physiological level of inflammation which is evident in the healthy gut may result from continuous challenge of the intestine by such organisms. It is also interesting to speculate that immune cells have a two-tiered level of response for commensal organisms, similar to that described above for pathogens. In this case, it may not be necessary to initiate responses specific for individual commensals but rather for major bacterial subdivisions. An example is the differential response of immune cells to Gram negative and Gram positive non-pathogenic organisms. Immune cells experience a more robust response to Gram-negative bacteria via TLR4 and other cell signalling pathways, than to Gram-positive bacteria via TLR2 (9). The overall response may be linked to the level of receptor expression. Certainly, the low expression of TLR2 and TLR4 on IECs *in vivo* limits the response to bacteria in the healthy intestine while a high expression may contribute to the clinical manifestations of IBD (11).

Appropriate regulation of both the innate and the adaptive immune responses to non-pathogens, may influence mucosal homeostasis (13). An example of the former is the hyporesponsiveness of mucosal IECs to the continuous presence of bacterial components (14). However, hyporesponsiveness or tolerance to bacterial products, typically endotoxin, is also observed in immunocompetent cells of the lamina propria. A lack of tolerance to bacterial products in the intestinal mucosa seems to be a central pathogenetic event in inflammatory bowel disease. Indeed, the types of genes that are associated with a susceptibility for IBD, suggests an abnormal recognition of conserved molecular motifs in bacteria which, in turn, leads to an inappropriate adaptive immune response that perpetuates tissue damage (15).

Although the systemic immune system in the neonate may be subjected to Th2-like default immune deviation, the mucosal immune system of the gastrointestinal tract and probably that of the respiratory tract, is up-regulated and manifests a more intense inflammatory reactivity than the physiological levels of the mature intestine (16).

Breast milk: a source of bioactive molecules that compensate for the functional deficits in bacterial recognition and immune homeostasis of the immature neonatal gut.

Breast-fed newborns experience a lower incidence of infections, inflammation and allergy than their formula fed counterparts. Breast feeding plays a dual function in the neonatal gastrointestinal tract (GIT). First of all, it improves protection against pathogens that are

attempting to colonise the sterile gut. This is achieved through breast milk factors such as immunocompetent cells, antibodies, anti-infectious oligosaccharides, lysozyme and lactoferrin. Second, immunomodulatory molecules present in milk modulate host reactivity during neonatal colonisation.

Molecules for bacterial recognition in the neonatal intestine: In addition to the aforementioned anti-infectious factors, we have postulated that milk contains factors which help the intestinal mucosa sense the presence of bacteria and initiate protective innate responses before bacteria can invade host cells (17). In so doing, tissue homeostasis is preserved. However, it is difficult to resolve why high luminal concentrations of sCD14, in the presence of an important bacterial inoculum, do not lead to an excessive and deleterious immune response. One possible explanation is that for mucosal sentinel cells (IECs, dendritic cells and lamina propria macrophages) to become hyporesponsive to commensals they need to have some level of activation for which pattern recognition receptors are needed. (18). Soluble forms of these receptors may therefore promote a hyporesponsive phenotype. Furthermore, it has been postulated that independently of its role in bacterial recognition, sCD14 can modulate T and B cell activation, and thereby sustain immune cell homeostasis (19, 20).

Molecules with a capacity to modulate the innate reaction to bacterial challenge.

Dysregulation of the responses which follow bacterial recognition can lead to inflammatory damage, shock and death. Indeed, during the neonatal period when mechanisms of immunological tolerance have not been consolidated, an exaggerated TLR-mediated response to bacteria may lead to necrotising enterocolitis and other less acute diseases that have an onset later in life. TLR2 mediates recognition of Gram-positive bacteria but through heterodimer formation with other TLRs, facilitates recognition of a wide range of microbial molecules (21). We have shown that soluble TLR2 (sTLR2) naturally occurs in human milk and serum and that is capable of modulating cell activation by bacterial lipopeptide (22). The exact mechanism by which this modulation is achieved is not clear however its interaction with sCD14 in the milk, may contribute to the inhibition observed.

Molecules with pleiotropic functions that modulate innate and adaptive immune responses.

Transforming growth factor- β (TGF- β) is a superfamily of cytokines which regulate many different cellular processes. TGF- β 2 is present in breast milk and can influence epithelial cell proliferation, differentiation and migration; the production of extracellular matrix and the modulation of the immune system and the inflammatory response.

TGF- β initiates signalling through ligand-dependent activation of a heterodimeric complex that comprises a transmembrane serine/threonine kinase TGF- β 1 receptor 1 and TGF- β 1 receptor 2. The signalling pathway involves Smad proteins. TGF- β 1 R1 phosphorylates Smad 2 and Smad 3 at the serine residue. Once activated Smad 2 and 3 associate with Smad 4 and translocate to the nucleus where they associate with the promoter regions of genes that downregulate immune/ inflammatory responses (23).

The multifunctional effects of TGF- β are such that transcription of many target genes in very different cell types is elicited. In the intestinal epithelium and in lamina propria macrophages, TGF- β can blunt the innate response to bacteria by down-regulating NF- κ B activation. In fact, it has been shown that TGF- β can increase I κ B α transcripts which can subsequently prevent the translocation of NF- κ B to the nucleus (24) and thereafter the activation of pro-inflammatory genes. Importantly, TGF- β is also the product of antigen-specific regulatory T cells that preserve oral tolerance.

The contribution of TGF- β to immune homeostasis and the activities of a naturally occurring TGF- β in bovine milk, prompted us to test the efficacy of a milk-based TGF- β containing diet

in the treatment of Crohn's disease (25). As anticipated, the diet decreased the levels of pro-inflammatory factors in both the circulation and the intestinal mucosa.

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