Bacterial colonization and the development of intestinal defences

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In humans, intestinal defences develop during gestation and, at full term, have the capacity to respond in an appropriate manner to infectious agents and foreign antigens. Before an active protective response can occur, however, the gut must first be exposed to colonizing bacteria. Colonization with diverse intestinal microbes is necessary for the development of important gut defenses such as the synthesis and secretion of polymeric immunoglobulin A and the generation of a balanced T helper (Th) cell response. Insights into normal immune physiological development of the gut have been made by studying the germ-free animal and intestinal defenses. These studies have provided insights into the physiology of immune responses. Two important immunological functions are the secretion of polymeric immunoglobulin A to protect the intestinal surface against harmful stimuli and inhibition of the systemic response to commensal bacteria and food proteins (eg, oral tolerance) to prevent chronic inflammation. Neither function exists in the germ-free state, but rapidly develops after conventionalization (colonization) of the germ-free animal.

In the present review, the importance of bacterial colonization on the appearance of normal mucosal immune function and to the clinical consequences of inadequate colonization to the development of disease will be discussed. For example, excessive Th2 activity can lead to atopy, whereas Th1 predominance is found in conditions such as *Helicobacter pylori* gastritis and Crohn's disease. With the eradication of infectious diseases in developed countries in the past three decades, the incidence of atopic and autoimmune diseases has increased. This epidemiological observation has been explained by the 'hygiene hypothesis', which suggests that a reduction in microbial burden by public health measures has contributed to an immunological imbalance in the intestine. A family of pattern recognition receptors (Toll-like receptors) on gut lymphoid and epithelial cells mediates innate immune responses to bacterial molecular patterns and, thereby, orchestrates acquired immunity. As the role of bacterial communication within the gut (bacterial-epithelial cross-talk) is clarified, physicians should be able to modulate gut immune responses, for example, by the use of probiotics.

Key Words: Bacterial-epithelial 'cross-talk'; Inflammatory bowel disease; Innate immunity; Intestinal allergy; Necrotizing enterocolitis; Toll-like receptors

Colonisation bactérienne et développement des mécanismes de défense dans l'intestin

Chez l'humain, les mécanismes de défense dans l'intestin se forment durant la gestation et, à terme, ils ont la capacité de réagir de façon appropriée aux agents infectieux et aux antigènes étrangers. Toutefois, avant que ne se produise une réaction active de protection, l'intestin doit d'abord avoir été exposé à des bactéries colonisatrices. La colonisation de l'intestin par diverses bactéries est nécessaire au développement d'importants mécanismes de défense comme la synthèse et la sécrétion d'immunoglobulines A polymériques et la production d'une réaction équilibrée des lymphocytes T auxiliaires (cellules Th). Des études sur animal axénique et sur les mécanismes de défense dans l'intestin ont permis de mieux comprendre le développement physiologique du système immunitaire et ses réactions. Celui-ci remplit deux fonctions importantes, soit la sécrétion d'immunoglobulines A polymériques pour protéger la muqueuse de l'intestin contre des agents dangereux et l'inhibition d'une réaction généralisée aux germes commensaux et aux protéines d'origine alimentaire (p. ex. tolérance buccale) pour prévenir l'inflammation chronique. Aucune de ces fonctions n'existe chez l'animal axénique, mais elles apparaissent rapidement après sa colonisation.

Il sera question dans le présent article de l'importance de la colonisation bactérienne pour le développement immunitaire normal des muqueuses et des conséquences cliniques d'une colonisation inadéquate dans l'apparition des maladies. Par exemple, une activité exagérée des cellules Th2 peut donner lieu à des manifestations atopiques, tandis qu'une prédominance des cellules Th1 est associée à des affections comme la gastrite à *Helicobacter pylori* et à la maladie de Crohn. Parallèlement à l'éradication des maladies infectieuses depuis une trentaine d'années dans les pays développés, la prévalence des réactions atopiques et des maladies auto-immunes a augmenté. La tendance s'expliquerait par une amélioration des conditions d'hygiène, hypothèse selon laquelle la diminution de la charge microbienne par des mesures de santé publique entraînerait paradoxalement un déséquilibre immunitaire dans l'intestin. Un groupe particulier de cellules épithéliales et de récepteurs de reconnaissance, les « toll-like receptors », se trouvant dans le tissu lymphoïde de l'intestin déclenche des réactions immunitaires naturelles à certaines structures moléculaires bactériennes, créant ainsi une immunité acquise. À mesure qu'on comprend davantage le rôle des bactéries dans l'intestin (échanges épithélium-bactéries), les médecins devraient être en mesure de modifier les réactions immunitaires de l'intestin en utilisant, par exemple, des probiotiques.

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Figure 1 A diagrammatic representation of the ontogeny of the human mucosal immune system, showing the major components of the mucosal immune system. The respective developmental time points for key components are highlighted. Initially, follicular epithelial cells (M cells) (1) are apparent. Note organized lymphoid tissue, such as Peyer's patches (2), and more diffusely scattered lymphocytes in the intestinal lamina propria (3) and intraepithelial lymphocytes (4) appear. This immune system is developmentally regulated. The development and function of the mucosal immune system can be affected by colonizing intestinal bacteria. sIgA Secretory immunoglobulin A

The intestinal mucosa is the interface between the external and internal environments, and separates gut luminal antigens, such as bacteria, bacterial products and food, from the largest population of lymphocytes in the body. At birth, the newborn infant leaves the germ-free intrauterine environment to enter a highly contaminated extrauterine world, which requires strong host defences to prevent the acquisition of gastrointestinal and systemic diseases. Intestinal defences develop during gestation, and at full term, have the capacity to respond in an appropriate manner to infectious agents and foreign antigens (1). However, before an active protective response can occur, the gut must first be exposed to colonizing bacteria. Colonization of the intestine with micro-organisms plays an important role in stimulating the normal development of the immune system, including the gut-associated lymphoid tissues (GALT), and important gut defences such as the synthesis and secretion of polymeric immunoglobulin A (IgA) and the generation of a balanced T helper (Th) cell response. Colonization begins as the newborn enters the birth canal, with exposure to maternal vaginal and colonic flora (2). Additional stimulation occurs with the introduction of enteric feeding (either breast milk or formula) and at weaning (3). Complete colonization with a large variety of organisms (more than 400 different species reside in the colon) occurs by two years of age. The number of microbes that ultimately colonize the gut exceeds the total number of cells in the body. Insights into normal immune physiological development of the gut have been made by studying the germ-free animal and intestinal defences. Animals housed in a germ-free environment show impaired development of the immune system and weak immune responses. The colonization of germ-free animals with intestinal commensal flora fosters the development of the immune system.

It is clear that there is a complex relationship between the intestinal immune system and commensal flora. Because

mucosal surfaces are colonized by a large, complex and dynamic collection of bacteria, it is extremely important for intestinal epithelial cells and the mucosal immune system to distinguish between pathogenic and nonpathogenic bacteria and to generate an appropriate response while maintaining a state of homeostasis with normal microbial flora. The cellular interactions between intestinal epithelial and immune cells are thought to be important for the initiation and regulation of the immune response to luminal bacterial antigens. Colonization of intestinal mucosal surfaces with normal intestinal flora is purported to be necessary for the development of tolerance to dietary antigens (4), whereas certain intestinal bacteria (probiotics) may be able to preferentially stimulate subsets of Th cells (Th1 and Th2) and, thereby, modify intestinal inflammatory and allergic responses (5). The present review discusses the role of intestinal bacterial colonization in regulating the development and function of the intestinal mucosal immune system and in the pathogenesis of intestinal diseases.

GALT

GALT are the largest collection of lymphoid tissues in the body. They consist of organized lymphoid tissues, such as mesenteric lymph nodes and Peyer's patches (PP), and more diffusely scattered lymphocytes in the intestinal lamina propria (LP) and epithelium (Figure 1), including large numbers of IgA⁺ plasmablasts.

Polymeric IgA, one of the key components of the intestinal humoral immune system, defends mucosal surfaces against environmental microbes. Germ-free mice have fewer than normal IgA-secreting plasmacytes (6). Monocolonization with segmented Gram-positive filamentous bacteria, a major constituent of the commensal flora, induced a robust mucosal IgA response in both PP and LP (7). It has been suggested that specific intestinal mucosal IgA can be induced by commensal bacterial antigens through a pathway that is independent of T cells and of the influence of follicularly organized lymphoid tissue (8).

It is well established that colonization with bacteria is critical to the normal structure and optimal function of the mucosal immune system (9). Early studies established that germ-free mice exhibit smaller PP (6) and fewer intraepithelial lymphocytes (10) than specific pathogen-free or germ-free mice colonized by single or multiple species of bacteria. A recent study (11) using gnotobiotic rats showed that, under germ-free conditions, the follicle-associated epithelium (FAE) contained only a few T and B cells, and that the B cells were negative for CD86, a co-stimulatory molecule necessary for T cell activation. After bacterial colonization, however, the number of T cells and CD86⁺ B cells in the FAE increased dramatically (11). Furthermore, this study showed that, before microbial colonization, immature (CD80⁻ or CD86⁻) CD4⁺ dendritic cell (DC) subsets were detected in the FAE. This subset disappeared from the FAE soon after colonization, probably as a result of rapid DC migration to the interfollicular T cell zone, where DC activation/maturation and stimulation of naive T cells are known to take place (Figure 2). The results of this study suggest the involvement of microbial colonization in organizing the spatial relationships between B cells, T cells and DCs in PP (11). Intestinal antigen-transporting M cells are also affected by luminal bacteria. Colonization of germ-free mice with *Salmonella typhimurium* aroA resulted in a two- to three-fold increase in the number of M cells (12).

One of the key functions of GALT is to distinguish innocuous antigens from pathogenic micro-organisms and to elicit an appropriate response. Intestinal microflora can significantly affect these responses. Intestinal bacterial antigens are believed to play a role in the activation of T cells because the majority of T lymphocytes from intestinal lymphoid tissues show an activated or memory phenotype. Monocolonization of germ-free animals can result in the activation of mucosal T cells that are otherwise quiescent. Unrestrained mucosal immune activation in response to bacterial signals from the lumen, however, is a risk factor for autoimmune disorders such as inflammatory bowel disease (IBD).

The development and function of GALT are precisely regulated by mechanisms that are still not fully understood. Regulatory T (Treg) cells may play a role in the modulation of mucosal immune responses. In the absence of Treg cells, gut bacteria trigger the development of severe IBD. An animal study (13) demonstrated that the transfer of CD4⁺ T cells to mice lacking the CD25⁺ subset led to colitis if the hosts were colonized by enteric bacteria. Adoptive transfer of Treg cells into these mice, however, prevented the expression of disease.

Immune responses to food proteins (allergy) or self-antigens (autoimmunity) may also result from failure to precisely regulate the mucosal immune system. Although intestinal T cells express an activated phenotype and are richly populated by memory cells, they proliferate poorly in response to *in vitro* T cell stimulation. Human mucosal CD4⁺ T cells have been shown to suppress the proliferation of peripheral blood T cells in response to antigen pre-pulsed antigen presenting cells (APC) in a co-culture system (14). This inhibition was independent of cell contact and was reversed by the neutralization of interleukin (IL)-10 or transforming growth factor-beta (TGF- β) with monoclonal antibody, suggesting that specific antigen recognition might play a role in maintaining mucosal T cell unresponsiveness to intestinal bacterial antigens. Germ-free animals have been shown to be partially or totally resistant to the induction of oral tolerance to some antigens (4,15-17). The priming of helper T cells in GALT by bacterial antigens might also help enable these cells to rapidly respond to the cognate antigen in peripheral lymphoid organs (18).

GALT can be divided into discrete inductive and effector sites, consisting of PP, LP and mesenteric lymph nodes, containing both CD4⁺ and CD8⁺ T cells. A subpopulation of CD4⁺ T cells that co-express CD25 (the α -chain of IL-2 receptor) exhibits a regulatory function in the mucosa and may be generated by exposure to luminal antigens. These cells appear to play an important role in the induction and maintenance of tolerance in normal intestine through the production of TGF- β and IL-10, and the ligation of cytotoxic T lymphocyte antigen 4 (19).

Treg cells, such as CD4⁺CD45RB^{lo} and CD4⁺CD25⁺, are potent immune suppressors that control or prevent the development of spontaneous autoimmune disease (20). They also prevent the excessive immune response to intestinal microflora that leads to IBD (21). A recent study (22) examined the role of microbial products in activating these regulatory cells and showed that Treg cells respond directly to proinflammatory bacterial products, including lipopolysaccharide (LPS). LPS treatment enhanced the suppressive function of these cells by interacting with the Toll-like receptor (TLR)-4 receptor molecule (22). In addition, this study also demonstrated that a subset of CD4⁺ cells

Figure 2 A diagrammatic representation of dendritic cell-microbial interaction. Dendritic cells in the lamina propria of the gut can sample luminal flora by extending appendages across tight junctions into the lumen. By interacting with pathogen-associated molecular patterns on Gram-positive and -negative bacteria via pattern recognition receptors (eg, Toll-like receptors [TLRs]), immature dendritic cells can mature to express surface molecules and release cytokines which favour naïve T helper (Th) cells maturing into various subclasses of Th cells (eg, Th1, Th2 and Th3). IL-10 Interleukin-10; INF- γ Interferon gamma; MHCII Major histocompatibility complex II; Treg Regulatory T cells

(CD45RB^{lo}CD25⁺) selectively express TLR4, TLR5, TLR7 and TLR8 (22), providing a link between the innate and adaptive immune responses.

BACTERIAL EPITHELIAL CROSS-TALK AND BACTERIAL ANTIGEN UPTAKE

Colonization of the gastrointestinal tract by normal bacterial flora is often cited as a basic defence mechanism of the body against infection by pathogens (23,24). Gnotobiotic mice or rats are much more susceptible than their conventionally reared counterparts to infection with *Salmonella enteritidis*, *Listeria monocytogenes*, *Clostridium difficile* and *Helicobacter pylori*. That neonatal animals and infants are more susceptible than adults to infections may be related to the change in the composition and density of gut flora after weaning. Alternatively, weaning may alter the gut flora, which may strengthen the mucosal immune system (23,25).

Colonization of the intestinal lumen begins at birth. The maternal microflora can be a source of bacteria for the newborn's intestine. Colonization can also be affected by environmental factors and infant feeding patterns. Breastfeeding encourages the growth of bifidobacteria, whereas formula-fed infants have a more complex flora made up of bifidobacteria, bacteroides, clostridia and streptococci. Bacteria may use microvillus membrane glycoconjugates as target cell receptors for adhering to the epithelium. The activities of glycosyltransferases, the enzymes responsible for synthesizing brush border glycoconjugates, are under tissue-specific and developmental regulation in mice (26). The intestinal flora has been shown to regulate specific glycosyltransferases (Figure 3) (26).

Even though intestinal epithelial cells are normally covered by a wide variety of microbes (up to 500 species of bacteria), they form a mechanical barrier that separates the external environment from the host's internal milieu. Intestinal epithelial cells are also capable of detecting bacterial antigens (or products) and initiating, participating in and regulating both innate

Figure 3) Cell surface glycoconjugates serve as adhesion sites for a variety of microbes. Glycosylation is a developmentally regulated process. Luminal bacteria are important extrinsic regulators of fucosyltransferases in the gut. In germ-free mice, the level of these glycosyltransferases maintain an immature pattern of surface glycosylation (**A**), whereas bacterial colonization initiates the developmental induction of fucosyltransferase (**B**). CONV Conventional; GF Germ-free; XGF Ex-germ-free

TABLE 1
Toll-like receptors (TLRs) and their ligands

TLRs	Ligands
TLR1	Triacyl lipopeptides
TLR2	Lipoprotein/lipopeptides, peptidoglycan, lipoteichoic acid, etc
TLR3	Double-stranded RNA
TLR4	LPS, HSP60, etc, commensal bacteria
TLR5	Flagellin
TLR6	Diacyl lipopeptides
TLR7	Synthetic compounds (the immune response modifiers)
TLR8	Unknown
TLR9	CpG-DNA
TLR10	Unknown

HSP Heat shock protein; LPS Lipopolysaccharide

and adaptive immune responses. They do this by transducing signals from luminal pathogens to adjacent immune cells in the LP, such as the macrophages, DCs and lymphocytes that form the intestinal mucosal immune system, via molecules expressed on the epithelial cell surface such as major histocompatibility complex I and II molecules, and TLRs (27-30). TLRs on epithelial cells may enable the epithelium to discriminate between normal commensal flora and pathogens, and induce the activation of a panel of genes mediating immune and inflammatory responses. Therefore, TLRs play an important role in signalling innate immune pathways, and in initiating and regulating adaptive immune responses. An imbalance or point mutation on these receptors can represent risk factors in conditions such as Crohn's disease (31).

TLRs are a family of transmembrane receptors homologous to *Drosophila* Toll proteins (32-34). Recognition of bacterial molecular patterns by the innate immune system is dependent on the interaction between TLRs and pathogen-associated molecular patterns (PAMPs) present on diverse microbes. To date, 10 members of the TLR family have been identified in mammals, with each receptor recognizing a unique set of PAMPs (Table 1). TLR2 is responsible for recognizing bacterial lipoproteins and peptidoglycan (PGN); TLR3 recognizes double-stranded RNA; TLR4 is essential for responses to LPS;

TLR5 controls responses to bacterial flagellin; TLR7 responds to the immune response modifiers (imidazoquinolines); and TLR9 is required for the recognition of unmethylated CpG DNA motifs characteristic of bacterial DNA. Recent studies (35) have suggested that TLRs may control the induction of adaptive Th1 responses. There is also evidence that TLRs may be important for Th2-type responses by augmenting the overall maturity of DCs (35).

The intestinal epithelium, which is continually exposed to a large variety of commensal bacteria and PAMPs, has been shown to constitutively express several members of the TLR family in vitro and in vivo (26-28,36,37). It is well established that the interaction of TLR with its PAMP results in the activation of intracellular signalling pathways, nuclear translocation of transcription factors, such as nuclear factor kappa B, and the transcription of cytokines, such as IL-8 (Figure 4).

Cario and Podolsky (28) characterized the expression pattern of TLR2, TLR3, TLR4 and TLR5 in intestinal mucosa of patients suffering from IBD and normal controls. Using immunohistochemistry, they observed a differential expression of TLRs in intestinal epithelial cells, in that there was constitutive expression of TLR2 and TLR5, and a low-level expression of TLR4 in normal cells. In contrast, intestinal epithelial cells from patients with IBD exhibited an upregulated expression of TLR4, downregulated expression of TLR3, and unchanged TLR2 and TLR5 expression (28). A study by Fusunyan et al (29) on a nonmalignant human fetal primary small intestinal cell line (H4 cells) and in human fetal small intestinal enterocytes showed that TLR2 and TLR4 were expressed constitutively, and that the transcription of TLR2 and TLR4 was regulated by inflammatory stimuli. The authors observed that IL-1 β stimulation resulted in an increased expression of both TLR2 and TLR4, whereas LPS decreased TLR4 expression in both H4 cells and human fetal small intestinal enterocytes. A study by the Sanderson group (38) showed that TLR4 messenger RNA (mRNA) was expressed in peripheral blood mononuclear cells, but was not present in epithelium from children with IBD.

The expression of TLRs in intestinal epithelial cells has also been studied in an animal model. Ortega-Cava et al (39) examined the localization of TLR4 compared with that of TLR2 and CD14 along the luminal gastrointestinal surface of

Figure 4) Enterocyte response to commensal bacteria in immature and mature cell lines (A). Because of the underexpression of signalling molecules (eg, inhibitory kappaB [IκB]) in immature cells, inflammatory cytokines are produced, in contrast to mature cells where anti-inflammatory responses predominate. **Panel B** demonstrates the interleukin-8 (IL-8) response in mature intestinal human cell lines (T-84) versus an immature human cell line (H4) to a pathological (SL3201) salmonella strain expressing flagella and to a commensal *Escherichia coli* (F18) strain expressing flagella. Immature enterocytes have an enhanced response to both pathological and commensal bacteria, in contrast to mature enterocyte response. H4 cells secreted a higher amount of IL-8 than T-84 cells in response to *salmonella and **E coli. NFκB Nuclear factor kappa B; P Phosphate; UBQ Ubiquitin

normal mice and mice with dextran sodium sulfate-induced IBD. They found that TLRs were differentially expressed along the mouse gastrointestinal tract, with higher levels of TLR4 and CD14 mRNA and protein detected in the distal colon, and stronger TLR2 mRNA expression in the proximal colon. The authors also observed that all genes studied (TLR2, TLR4 and CD14 MyD88) were upregulated during dextran sodium sulfate-induced inflammation, which is localized to the distal colon (39).

To understand how the normal intestinal epithelium deals with continuous exposure to commensal Gram-positive bacteria (which possess PAMPs, including PGN and bacterial lipopeptides that are recognized by TLR2), Melmed et al (37) isolated human colonic epithelial cells by laser capture microscopy and used intestinal epithelial cell (IEC) lines (Caco-2, T84 and HT-29), and reverse transcriptase polymerase chain reaction (PCR) and quantitative real-time PCR to analyze the expression of TLR2, TLR6, TLR1 and Toll inhibitory protein mRNA. They found that colonic epithelial cells and LP lymphoid cells from both healthy and inflamed tissue expressed low levels of TLR2 and high levels of the Toll inhibitory protein, and that IECs were unresponsive to TLR2 ligands. The observation that IECs expressed a low level of TLR2 was consistent with previous observations by Cario et al (27) and by the Sanderson group (38) that TLR2 mRNA was highly expressed in peripheral blood mononuclear cells and was present in all human IECs. These results indicate that TLRs expressed on intestinal epithelium are regulated by bacterial flora and host mucosal immune responses. TLRs on IECs may play a role in the induction of an inflammatory response to bacterial pathogens and in the development of tolerance to non-pathogenic commensal organisms.

Intestinal bacteria, especially pathogenic ones, are believed to be taken up and actively translocated by specialized epithelial cells, M cells, which are derived from villous epithelial cells located over PP. A study by Rescigno et al (40) found that DCs, which are crucial to the recognition of bacterial antigens

and in the defence against pathogens, expressed tight junction protein and could open the tight junctions between epithelial cells and, thus, take up bacteria directly without compromising epithelial barrier function. As an initiator of adaptive immune responses, mucosal DCs either infiltrate the intestinal LP diffusely or form subepithelial aggregates scattered along the small and large intestine (41).

DCs are activated via TLRs expressed on their surface. Liu et al (42) observed that myeloid and lymphoid subsets of DCs express different TLRs. Specifically, human myeloid pre-DC1s, but not lymphoid pre-DC2s, express TLR2 and TLR4, whereas lymphoid pre-DC2s, but not myeloid pre-DC1s, express high levels of TLR7 and TLR9. The activation of TLRs induces DC expression of various cytokines, such as tumour necrosis factor-α (TNF-α), IL-6 and IL-12. IL-12 can induce Th cells to differentiate into Th1 effector cells (42). Recent evidence has revealed differences in TLR expression on murine DC subsets. Reverse transcriptase-PCR has been employed to detect the mRNA of most TLRs, including TLR1, TLR2, TLR4, TLR6, TLR8 and TLR9, on all DCs. A difference in TLR3, TLR5 and TLR7 expression was found between CD8α⁺ and CD8α⁻ DC subsets (43). There are also strain-specific differences: TLR2, TLR4, TLR5 and TLR6 are strongly expressed on DCs from BALB/c mice, but TLR9 is preferentially expressed in C57BL/6 mice (44). The functional significance of these differential expressions of TLRs on DCs and DC subsets remains to be further elucidated.

TLRs expressed on DCs constitute a critical link between antigen recognition and the induction of T cell immunity. Triggering of TLRs by various microbial stimuli may drive DCs to assume distinct phenotypes and functions. Using LPS (TLR4 ligand) and PGN (TLR2 ligand), Re and Strominger (45) showed that human DCs preferentially express IL-12 and IL-10 when stimulated through TLR4 and TLR2, respectively. Similar observations were made in an animal study (46) that demonstrated that LPS-stimulated DCs produced high levels of IL-12 and low levels of IL-10, whereas DCs exposed to PGN

produced low levels of IL-12 and high levels of IL-10. On the other hand, Higgins et al (47) recently showed that signalling through TLR4 in response to *Bordetella pertussis* activates IL-10 production from DCs, suggesting that the TLR4 pathway may promote the development of IL-10-secreting Treg cells, thereby suppressing the inflammatory response. It is clear that pattern-recognition receptors on IECs and APCs play an important role in mediating the continuing dialogue between the host and gut microbiota, and in generating the appropriate response.

BACTERIAL COLONIZATION AND DISEASE

Because the gut mucosa is in continuous, direct contact with the intestinal flora, the GALT must develop mechanisms to distinguish between pathogens and commensal flora, and determine whether tolerance or an immune response should be induced. A breakdown of the tightly regulated mucosal immune response may result in inappropriate reactivity of the intestinal mucosa against luminal antigens. In fact, a triggering role of intestinal bacteria in chronic intestinal inflammation has been suggested by both clinical observations and animal experiments. A large concentration of mucosal bacteria was detected in patients with IBD but not in healthy controls (48), suggesting that a healthy mucosa may prevent close contact of the luminal flora with the epithelial surface. It is known that murine lymphocytes do not proliferate when stimulated with bacterial antigens from their own intestinal flora, indicating the development of immunological tolerance.

Increased mucosal permeability, often occurring with IBD, allows the entry of bacterial antigens. The interaction of certain commensal bacteria with the mucosa triggers mucosal inflammation. The gut flora is, therefore, the key factor driving the inflammatory process that leads to intestinal lesions. Both the innate immune system and Treg cells likely regulate the mucosal immune system.

Abnormalities of the intestinal microflora early in life may also lead to immune disturbances. Intestinal micro-organisms induce a Th1 response that may balance Th cell responses in the mucosa. Delayed or aberrant colonization early in life may result in a reduced capacity of APCs to provide pro-Th1 signals, such as IL-12, to T cells, leading to the development of an unbalanced Th2 response and allergic reactions. A recent study by Eggesbo et al (49) considered factors that possibly delay or alter initial bacterial colonization, and discovered a sevenfold greater incidence of food allergy among children delivered by cesarean section. It was noted that the risk was especially high among those with a familial predisposition.

Bacterial colonization at birth can be affected by many factors, including the composition of the maternal vaginal microflora. Benn et al (50) recently found that maternal colonization with *Ureaplasma urealyticum* during pregnancy was a risk factor for subsequent infant wheezing, and that maternal colonization with staphylococci and the use of antibiotics during pregnancy were associated with asthma during the first five years of life.

Epidemiological studies in countries with different prevalences of atopic diseases (Sweden and Estonia) have suggested an association between normal gut flora and the development of allergies (50,51). Children with allergies are less often colonized with lactobacilli and have higher counts of aerobic bacteria than nonallergic children. Early colonization with bifidobacteria, and low counts of bacteroides and *C difficile* also

appear to be protective against allergy (52). These observations support the 'hygiene hypothesis', which suggests that inadequate exposure to bacteria favours the development of allergic disease (53).

PROBIOTICS AND GUT DEFENCES

As already discussed, colonization of the intestine, especially by certain bacteria, may contribute to the induction and maintenance of immune tolerance to luminal antigens and help prevent autoimmune disease and food allergies. There is recent evidence that commensal bacteria affect intestinal development and function (52). Probiotics are live micro-organisms that are ingested to enhance health by altering indigenous microflora.

The beneficial effects of probiotics may be related to a variety of mechanisms: the regulation of cytokine production, increased release of IgA secretion, production of anti-bacterial substances, augmentation of tight junctions to prevent intercellular bacterial invasion and competition with pathogenic micro-organisms for adherence to enterocytes. A recent study (54) found that one probiotic species, *Lactobacillus rhamnosus* GG, promoted the survival of intestinal epithelial cells through the activation of antiapoptotic Akt/protein kinase B and inhibition of proapoptotic p38 mitogen-activated protein kinase pathways. The results of this study provide new insights into the understanding of the signal transduction pathways in enterocytes that are regulated by probiotics and intestinal commensal bacteria.

Lactobacilli derived from the endogenous flora of normal donors have been used as probiotics in medical foods and as vaccine stimuli. Probiotic treatment has been used to prevent some infectious and allergic diseases, and may be effective for IBD. Growing evidence from human studies and animal models of IBD implicate an aberrant response to altered enteric flora (48,55). There is also evidence from a murine IL-10 knockout model that demonstrated microflora disturbances may promote intestinal inflammation (55). IL-10-deficient mice exhibited increased adherence and translocation of aerobic bacteria, even before the development of overt colonic injury. Decreased adherence of *Lactobacillus* species occurred during the time when histological injury was developing. Repopulation of the intestinal lumen *Lactobacillus* species attenuated the disease.

In a recent double-blind, placebo-controlled trial, McCarthy et al (56) found that both *Lactobacillus salivairus* and *Bifidobacterium infantis* significantly attenuated colitis in IL-10-deficient mice. The investigators found that PP lymphocytes from *B infantis*-treated mice produced significantly less interferon-gamma and TNF- α . There was also a significant reduction in proinflammatory cytokine production by spleen cells in the probiotic groups. However, the production of the immunoregulatory cytokine TGF- β remained unchanged, suggesting that the probiotic effect might be mediated by altering the balance between proinflammatory and regulatory cytokines (56). It is possible that specific luminal micro-organisms could induce the production of IL-10 and TGF- β , which play important regulatory roles in the development of inflammatory and allergic responses, and in the induction of oral tolerance.

Borruel et al (57) collected samples of intestinal mucosa from 10 patients with Crohn's disease and five disease controls undergoing right hemicolectomy, and cultured the mucosa with

nonpathogenic *Escherichia coli*, *Lactobacillus casei*, *Lactobacillus bulgaricus* or *Lactobacillus crispatus*. They found that the production of the proinflammatory cytokine TNF- α by inflamed Crohn's disease mucosa was significantly reduced by co-culture with *L. casei* or *L. bulgaricus*, suggesting that probiotics may interact with immunocompetent cells at the mucosal interface and modulate the local production of proinflammatory cytokines.

Previously, in a double-blind, randomized, placebo-controlled trial, Kalliomäki et al (58) gave *Lactobacillus* GG prenatally to mothers, who had at least one first-degree relative (or partner) with allergy (atopic eczema, allergic rhinitis or asthma), and postnatally to their infants (Figure 5). They found that probiotic treatment significantly reduced the incidence of atopic eczema. At follow-up at four years of age, a diagnosis of atopic eczema (based on a questionnaire and clinical examination) was established in 14 of 53 children receiving *Lactobacillus* GG compared with 25 of 54 children receiving placebo (59). This observation suggests that the preventive effect of *Lactobacillus* GG on atopic eczema extends beyond infancy.

Lodinova-Zadnikova et al (60) reported that colonization of the intestine with a probiotic form of *E. coli* after birth resulted in a significant decrease in the incidence of allergies and repeated infections both 10 and 20 years later. Intestinal flora may also promote potentially antiallergenic processes by inducing Th1-type immunity. The gut microflora can play a suppressive role in the development of a Th2-skewed immune response in fetuses and neonates.

SUMMARY

Bacterial colonization plays an important role in the normal development, differentiation, function and regulation of the intestinal mucosal immune system. Through mechanisms that are still not fully understood, the intestinal mucosal immune system generates protective immunity against pathogen invasion and, at the same time, induces immune tolerance, preventing the development of autoimmunity (IBD) and food allergy. The regulatory role of the intestinal flora in the development and function of the intestinal mucosal immune system is well established. Recent work has suggested that colonization with probiotics in the gut may play an essential role in balancing the functions of Th1, Th2 and Th3 cell populations. This action may contribute to the induction and maintenance of immunological tolerance to

Probiotics and allergy prevention (2yrs)

Figure 5) Atopic response in allergy prone infants treated in utero and during the first six months of life with *Lactobacillus* GG versus placebo. The infants treated with probiotics had 50% less atopic dermatitis at two years of age than did infants treated with the placebo (Reproduced with permission from Kallimaki et al (58). yrs Years

other luminal antigens in the normal host or to the inhibition of dysregulated responses induced by luminal antigens in diseased hosts. A better understanding of the cellular and molecular mechanisms controlling the development and regulation of the intestinal mucosal epithelial system by intestinal bacteria (commensal and probiotics), and their regulatory role in various diseases will help establish new strategies to prevent and control these conditions.

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