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Intestinal Colonization and Programming of the Intestinal Immune Response

W. Allan Walker, M.D.

Abstract

Initial bacterial colonization of the gut is a vital component of the development of the gastrointestinal tract, particularly mucosal immune protection, during the neonatal period. Newborn infants in their protected intrauterine environment are suddenly thrust into a highly contaminated extrauterine state. Although mucosal host defenses have developed *in utero* during fetal maturation due to the stimulation of ingested trophic factors in amniotic fluid, actual active protection only occurs when colonizing bacteria stimulate the gut mucosal barrier. Colonization evolves over a period of about one year and is dependent on the mode of delivery, use of perinatal antibiotics, age at birth and infant feeding. A fully colonized gut consists of 10^{14} bacteria, establishes a symbiotic relationship with the host and insures normal development and immune homeostasis. Colonizing bacteria can also affect the epithelial mucosal barrier and the innate and adaptive immune systems. Disruption of normal colonization, dysbiosis, is associated with increased expression of disease. Evidence exists that the use of probiotics with dysbiosis may prevent disease expression.

Keywords

intestinal colonization; intestinal defenses; probiotics

INTRODUCTION

Over the last few decades, we have become aware of the importance of initial bacterial colonization in the development of the gastrointestinal tract, particularly mucosal immune protection, during the neonatal period. Appropriate colonization stimulates the maturation of immune defenses to provide protection for the newborn in the extrauterine environment. Normal initial colonization is affected by numerous environmental factors (birth process, use of antibiotics, age of delivery, nature of initial feeding). Under ideal conditions, a full term infant born by vaginal delivery without antibiotics and exclusively breast fed appropriately colonizes the gut in stages over the first year of life. With complete colonization, an individualized, genetically-defined signature of bacteria unique to that individual is established which principally colonizes the distal small intestine and colon with 10^{14} organisms. This microbiome is made up of balanced phyla (large families of bacteria)

Corresponding Author: W. Allan Walker, M.D., Conrad Taff Professor of Nutrition, Professor of Pediatrics, Harvard Medical School, Investigator, Mucosal Immunology and Biology Research Center, Division of Pediatric, Gastroenterology, Hepatology and Nutrition, MassGeneral Hospital for Children, Boston, MA, Tel: 617-726-7991, Fax: 617-726-4172, wwalker@partners.org.

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and contains about a thousand different species of bacteria. These microbes are said to make up an ancillary body organ which establishes a symbiotic relationship with the human host via expressed molecules on the surface of bacteria or as secreted product of bacteria into the intestinal lumen. These symbiotic bacteria communicate with intestinal epithelial and lymphoid cells via pattern recognition receptors, e.g., toll-like receptors (TLRs), which trigger cellular signaling, activation of transcription factors and translation of cytokines, chemokines, etc. that mediate the immune response, enterocyte development and mucosal barrier function. This review will briefly consider how colonizing bacteria stimulate programming of the intestinal immune response.

SYMBIOSIS AND IMMUNE FUNCTION

The interaction of symbiotic commensal bacteria with the gut during colonization impacts on development of important mucosal barrier epithelial functions (FIGURE 1) (1). For example, colonizing bacteria can stimulate the release of mucus from goblet cells to coat the intestinal surface as a glycocalyx which contributes an added protective barrier. In addition, commensal bacteria can stimulate the epithelial transcription and translation of tight junction proteins to close the intercellular route for the uptake of large molecules and microbiota. Furthermore, intestinal microbiota can stimulate epithelial cells and Paneth cells to release anti-microbial molecules (defensins) to provide an additional protection against pathologic bacterial penetration.

In like manner, colonizing bacteria through its symbiotic relationship with gut immune cells can stimulate both innate and adaptive immunity. Intestinal epithelial cells, dendritic cells and lymphocytes express pattern recognition receptors on their cell surface and within their cytoplasm. Molecular patterns expressed on the bacterial cell surface or secreted into the intestinal lumen can stimulate these pattern recognition receptors to evoke a self-limited, acute innate inflammatory response to prevent pathogen penetration of the epithelium (2). The most studied pattern recognition receptors are the toll receptors TLRs. Gram negative bacteria through lypopolysaccarhide (LPS) on their surface can interact with TLR-4 receptors evoking a signaling transduction response leading to a cytoplasmic release of NFκB, a transcription factor, which enters the nucleus and interacts with chromosomes to stimulate the transcription and eventual translation of the chemokine IL-8 which in turn attacks the migration of neutrophils to the site of pathogen interaction. In addition, inflammatory cytokines such as TNF-α are released to activate an inflammatory response to certain pathogens. To create a self-limited inflammatory response and not chronic inflammation, sustained interaction of LPS with TLR-4 activates additional molecules that inhibit the NFκB activation response (LPS tolerance). In like manner peptidoglycans expressed on gram positive organisms can interact with TLR-2 to invoke a self-limited inflammatory response to contain pathogen access to the blood stream (3).

Recent publications (4,5) have shown a close association between the innate and adaptive immune responses mediated by colonizing commensal bacteria. Dendritic cells underlying the intestinal epithelium can separate tight junctions and penetrate its appendage expressing TLRs into the lumen. Interaction of molecular patterns and colonizing bacteria with these expressed TLRs on dendritic cells can invoke a release of cytokines, etc. that create a

microenvironment that allows naïve T-helper cells (Th0) to differentiate into a balanced Th sub-class response, e.g., Th1, Th2, Th17 and Tregs (FIGURE 2). This interaction leads to intestinal immune homeostasis and no expression of disease. In the absence of adequate initial colonization, a dysbiosis occurs and there is an increase in immune-mediated diseases (allergy, autoimmune disease including type 1 diabetes).

A major development of mucosal immune homeostasis involves the capacity of T-helper cells to produce tolerance to innocuous antigens and bacteria. Tolerance can not occur in the absence of intestinal colonization (6) and is most effective if colonization occurs in the newborn period (7). Recently it has been shown that breast feeding as the first source of oral feeding for the neonate has an effect on the nature of expression of health promoting bacteria (8). Specific organisms activated by factors in breast milk preferentially stimulate an increase in *Bacteroides, Bifidobacteria* and *Lactobacillus* organisms. These organisms have important specific effects on neonatal immune function. For example, a polysaccharide (PSA) expressed on a *Bacteroides fragillis* species can interact with the TLR-2 receptor on dendritic cells and preferentially create a milieu that stimulates Treg cells (9). Other organisms such as *Clostridial* species can also provide the same effect. In like manner, *Bifidobacteria infantis* can preferentially activate B cells to mature into pIgA producing plasma cells (10). These immunoglobulins coat the intestinal surface and help protect against pathogen penetration.

INFANT NUTRITION AND COLONIZATION

An important environmental factor in intestinal colonization is the nature of the diet. We know that specific diets in adults over long periods of time evoke the large families of intestinal bacteria which act as functional units called enterotypes (11). We also know that the composition of intestinal bacteria in African children living in a primitive setting and ingesting a high fiber complex carbohydrate diet compared to age-matched children in Florence, Italy ingesting a typical Westernized diet with processed food and animal fat and protein is strikingly different (12). Since we know that the composition of intestinal bacteria influences the phenotypic expression of disease, this observation may explain the considerable difference between the disease burden in these two child populations.

At no time in the children's life is nutrition, as an environmental factor for bacterial colonization more important than with initial feeding at birth at the time of the first colonization. More specifically, as mentioned breast fed infants have a different intestinal microbiota than formula fed infants (13). Not only are health-promoting bacteria (*Bifidobacteria, Bacteroides, Clostridial* and *Lactobacillus*) present early with breast feeding, but the genes produced by these organisms have a profound effect on bacterial components that are secreted and in turn an effect on the enterocyte's response with regard to development and protection (14). Several factors in breast milk (non-metabolized oligosaccharide, lysozyme, lactoferrin, antibodies and cytokines) alter the intestinal milieu to stimulate specific indigenous bacteria to proliferate (15).

DYSBIOSIS AND DISEASE

As mentioned before, specific disease states are associated with a dysbiotic microbiome different from their age-matched normal controls (FIGURE 3) (16). Studies transferring the intestinal microbiome from patients with clinical conditions (obesity, diabetes, etc.) or animal models of autoimmune disease to germ-free animals cause these animals to express the disease suggesting that intestinal microbiota at the very least function as an intermediary in disease expression. Although not proven some investigators suggest that microbiota may be the actual cause of disease.

There are conditions in which the phenotypic expression of disease associated with a dysbiosis may be improved with probiotics. Unfortunately most of the reported conditions have not been subjected to a multi-center, single protocol, double blind study which is necessary and in whose absence weakens the conclusions, even with strong evidence by meta-analysis support. One condition with a known dysbiosis is necrotizing enterocolitis (NEC) which occurs in less than 1500 gms premature at an incidence of between 7 and 12%. These studies have shown that several known probiotics can help to prevent or lessen the severity of NEC and several meta-analysis are supportive. One multi-center study done in Taiwan showed that a combination of *Bifidobacteria infantis* and *Lactobacillus acidophilus* given with expressed breast milk from the birth mother was effective (17). We have shown that a 30 kD glycan secreted from *Bifidobacteria infantis* is anti-inflammatory in human *in vitro* intestinal models (18) and *in vivo* in a mouse model of newborn inflammation (19). We plan a clinical trial with a specific protocol in premature newborns after an appropriate safety studies are done.

SUMMARY AND CONCLUSIONS

Extensive studies over the last decade have shown the importance of intestinal colonization in gut function, particularly protective function. The importance of initial colonization is emphasized since the process has a profound effect on the development of mucosal defenses leading to immune homeostasis and the decreased expression of neonatal infectious diseases and immune-mediated disease states later in life. Breast feeding is an important environmental determinant of normal colonization during infancy. Dysbiotic states leading to diseases, e.g. NEC in prematures, may be prevented by aggressive use of probiotics.

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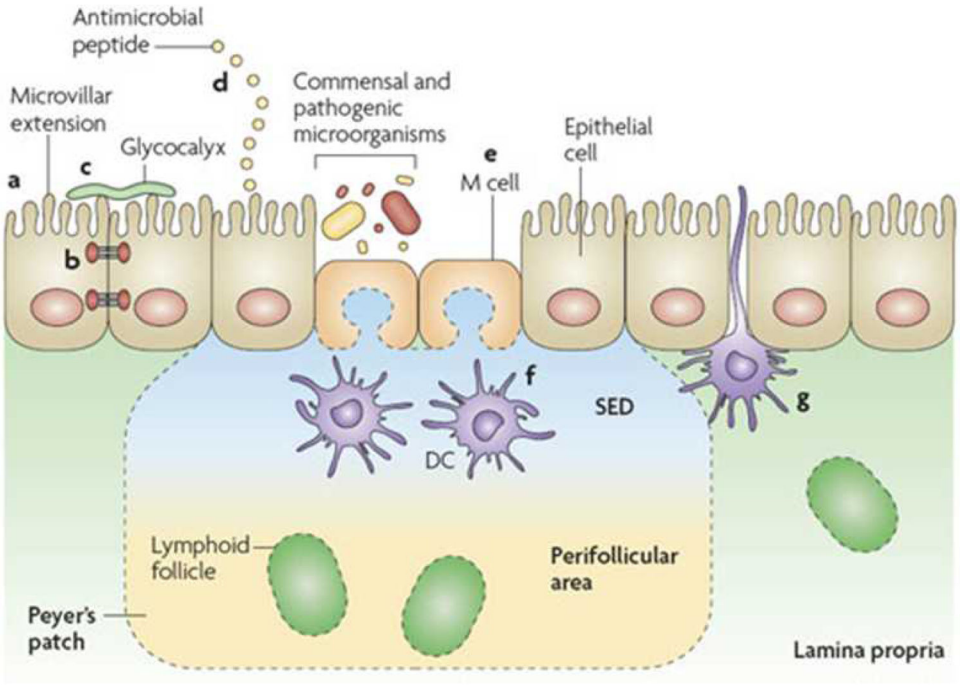

Figure 1.

The intestinal epithelial-cell barrier. Simple columnar epithelial cells exhibit physical and biochemical adaptations to microbial colonization to maintain barrier integrity including actin-rich microvillar extensions **(a),** epithelial-cell tight junctions **(b),** apically attached and secreted mucins that form a glycocalyx **(c)** and the production of various anti microbial peptides **(d)**. Specialized intestinal epithelial cells known as M (microfold) cells overlie Peyer's patches and lymphoid follicles to facilitate luminal sampling. M cells exhibit reduced mucin secretion and have modified apical and basolateral surfaces **(e)** to promote uptake and transport of luminal contents to professional antigen-presenting cells that inhabit the subepithelial dome (SED) of the Peyer's patches and lymphoid follicles **(f)**. Specialized dendritic cell (CD)subsets can also extend dendrites between the tight junctions of intestinal epithelial cells to sample luminal contents **(g)**. (Reproduced with permission from reference 1.)

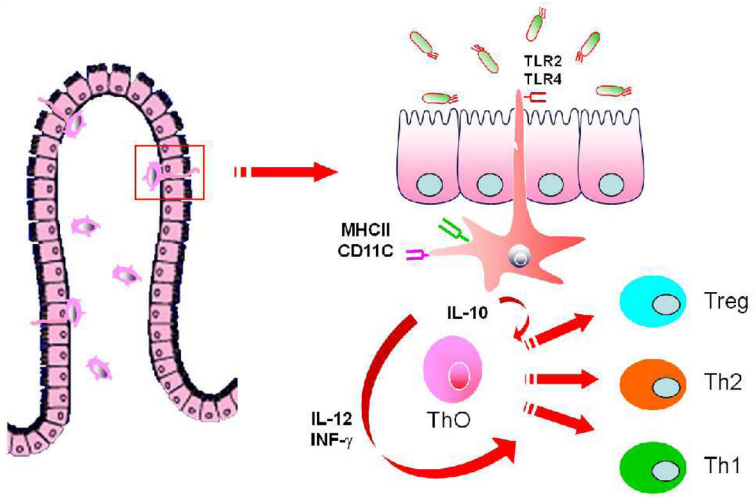

Figure 2.

Cartoon depicting sub-epithelial dendritic cells extending their appendages between enterocytes into the intestinal lumen. These appendices, displaying TLRs, are activated by colonizing bacteria, mature and secrete cytokines which activate naïve T cells (TH0) to mature into a balanced T helper cell response (Th1, Th2, Th17 and Treg). (Reproduced with permission from reference 20.)

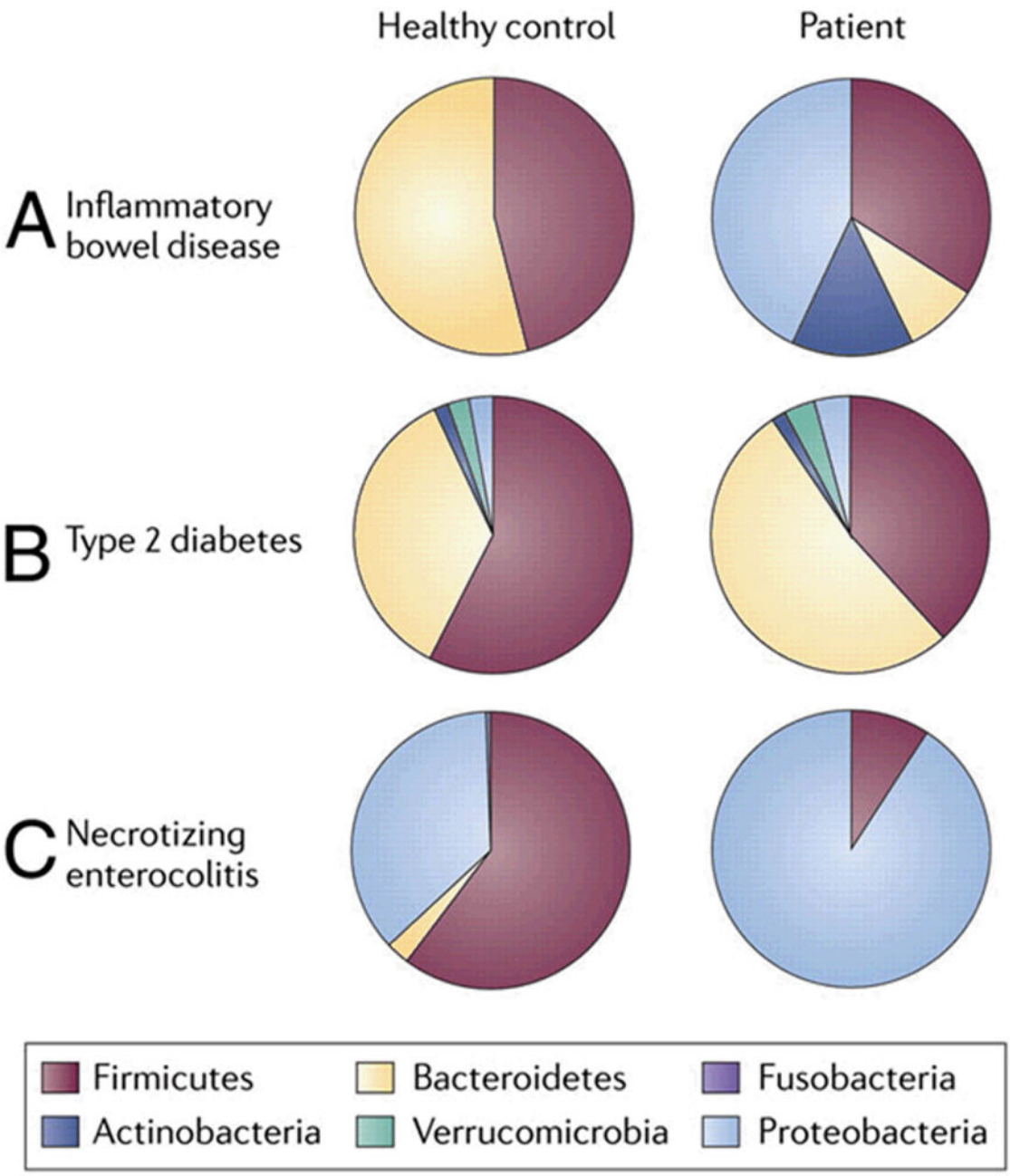

Figure 3.

Disease states reveal phylum-level differences compared with healthy controls. Comparisons of the relative abundances of predominant bacterial phyla in IBD, type 2 diabetes, and NEC compared with healthy controls. Fecal samples from infants with NEC and patients with type 2 diabetes were compared with healthy controls revealing a predominance of Proteobacteria in patients with NEC. Cecal samples from patients with IBD were compared with healthy controls, and relative abundances were assessed. (Reproduced with permission from reference 16.)