

Immunophysiology and Nutrition of the Gut

Samuli Rautava, MD, PhD
W. Allan Walker, MD

INTRODUCTION

The intestinal surface comes into contact with a continuous flux of a vast variety of antigens. The primary function of the intestinal mucosa is absorption of nutrients. This function necessitates the transport of molecules across a single layer of epithelial cells and distinguishes the intestinal mucosa functionally from that of the respiratory or genitourinary tracts. The intestinal immune system must be able to recognize potential pathogens and inhibit their adherence and invasion by protective immune exclusion or inflammatory responsiveness while allowing uptake and transport of dietary compounds and certain intact macromolecules (eg, growth factors and maternal immunoglobulins in breast milk) without inappropriate and detrimental immune reactions. Appreciation of this intricate balance is paramount to our understanding of intestinal immunophysiology.

The intestinal immune system has unique have evolved to fulfill the dual task of immune defense and tolerance. An overview of the intestinal immune system with emphasis on immune responses directed toward dietary antigens is provided in the first half of this chapter. The establishment and maintenance of nonresponsiveness or tolerance to dietary and commensal microbial antigens is the result of active and coordinated immune responses which mature postnatally upon contact with foreign antigens, particularly colonizing bacteria. The indigenous intestinal microbiota is established during the same period and commensal bacteria have a profound impact on the structural and functional maturation of the gut. Indeed, the intestinal microbiota may be considered an essential component of the intestinal immune system (Figure 1).

The second half of this chapter is dedicated to the effects of nutritional factors to gut immune physiology. Malnutrition is still a major threat to infant and child health in large portions of the world. The effects of protein and energy malnutrition as well as the lack of certain individual nutrients on gut immunophysiology are overviewed in this chapter but discussed in detail in Chapter 8, “Trace Elements,” Chapter 9, “Iron,” Chapter 10, “Vitamins,” and Chapter 13, “Protein-Energy Malnutrition: Pathophysiology, Clinical Consequences, and Treatment.” The increasing evidence from clinical and experi-

mental studies indicating that the route of nutrition, that is, enteral versus parenteral nutrition, affects intestinal immune functions and therefore child health is then discussed. In the final paragraphs, an overview of novel nutritional approaches aiming to enhance intestinal and systemic immune functions, namely probiotics and prebiotics, is given.

IMMUNE MONITORING OF INTESTINAL ANTIGENS

The Intestinal Immune System

The intestinal immune system must be able to recognize potential pathogens and launch protective immune responses in order to avoid invasion by microbes. The innate immune system consists of evolutionarily conserved immune defense mechanisms which provide the host with immediate antimicrobial protection. These include soluble factors such as lysozyme, peroxidase, and lactoferrin as well as phagocytic immune cells, most importantly macrophages and neutrophilic granulocytes. Pattern-recognition receptors (PRRs) such as cell membrane-bound Toll-like receptors (TLRs) and cytosolic nucleotide-binding oligomerization domain (NOD) molecules identify specific molecular patterns characteristic to microbes and initiate rapid innate immune responses. The common characteristic of innate immune responsiveness is generally thought to be the lack of immune memory; that is, innate responses remain unaltered upon reencounter with the same antigen.¹ However, it is also well established that exposure to microbial antigens can increase or decrease the expression of

PRRs in vitro and in vivo.^{2,3} Moreover, the innate immune system controls adaptive immune responses through PRR signaling and activation of antigen-presenting cells (APCs).^{4,5}

Adaptive or acquired immune responses are by definition characterized by antigen-specific immune memory. Induction of adaptive immunity is considerably slower than that of innate immunity but upon subsequent challenge with the same antigen a prompt and powerful specific immune protective response is elicited. Adaptive immunity is characteristic of vertebrate animals and its evolutionary development intriguingly coincides with the appearance of other important structural and functional organ changes including the appearance of the lower jaw.⁶ In addition to its role in defense against potential pathogens, immunological memory is essential for avoiding inappropriate inflammatory reactions against dietary antigens and commensal microbes. It may therefore be suggested that intestinal immunophysiology necessitated the development of an adaptive immune system and, indeed, the intestinal immune system comprises intraepithelial lymphocytes and organized gut-associated lymphoid tissues is the largest lymphoid organ in the body. The functions of the intestinal adaptive immune system range from production and secretion of polymeric immunoglobulin IgA antibodies in the gut lumen to induction of protective immune responses which may then be elicited locally, systemically, or at other mucosal effector sites such as the mammary gland.

Intestinal Mucosal Barrier

The intestinal immune system must be able to monitor luminal antigens in order to prevent potential pathogens from adhering and invading the epithelium and to neutralize microbial toxins. Immunophysiological factors that restrict mucosal colonization by pathogens, prevent foreign noxious antigens and pathogens from penetrating the mucosa, and regulate antigen-specific immune responses are collectively referred to as the intestinal mucosal barrier (Figure 2).⁷ To carry out the primary function of nutrient uptake in the gut, however, these protective mechanisms must also allow the controlled entry of certain antigens. Permeability of the gut barrier is increased during the neonatal period, which may have a physiologic role in allowing the uptake of growth factors and maternal IgG antibodies from breast

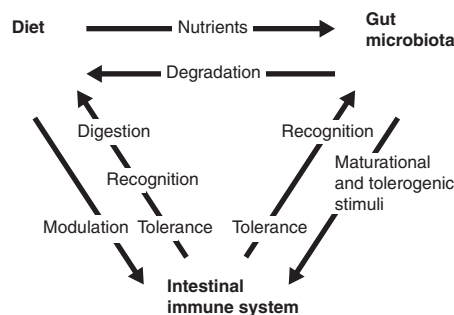


Figure 1 The interaction between diet, the indigenous intestinal microbiota, and the intestinal immune system.

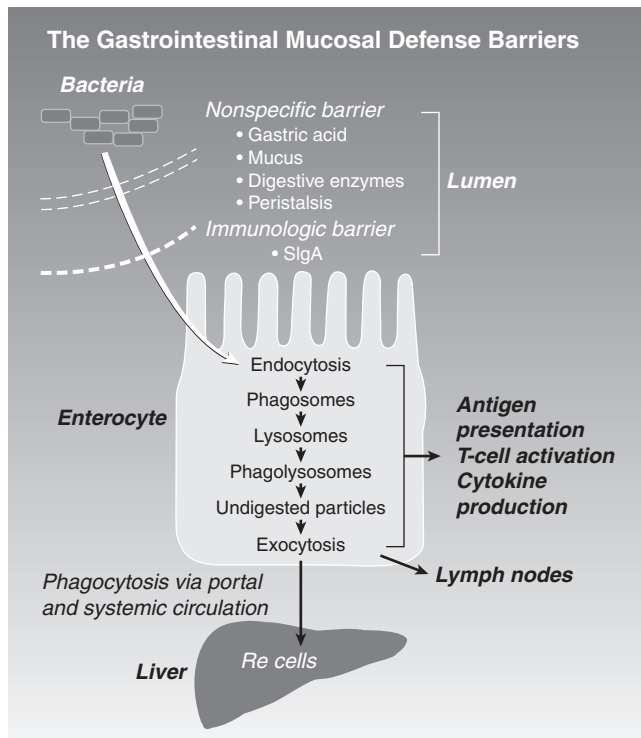


Figure 2 The intestinal mucosal barrier selectively restricts mucosal pathogen colonization and invasion and prevents foreign antigens from penetrating the mucosa. While nutrients such as glucose and amino acids are absorbed through the apical cell membrane and traverse the enterocyte to exit at the basolateral membrane, macromolecules may bind to specific receptors on the enterocyte and then be engulfed by the cell in membrane-bound vesicles invaginating from the apical cell membrane. The endocytosed antigens may then be directed to lysosomes and destroyed or released on the basolateral border of the enterocyte as in the case of maternal IgG antibodies and growth factors from breast milk. Moreover, presentation of intact antigens to T lymphocytes of the adaptive immune system is necessary for the induction of protective sIgA production and the establishment of oral tolerance. sIgA = secretory immunoglobulin A.

milk. On the other hand, this relative intestinal permeability may render the neonate susceptible to pathogen invasion and allergen sensitization. A compromised gut barrier function is also characteristic of pathological conditions such as necrotizing enterocolitis (NEC) and inflammatory bowel disease (IBD).^{8,9}

External Mucosal Barrier. The intestinal mucosal barrier may be considered to be composed of four major components (Table 1). It should be born in mind, however, that these components are structurally and functionally interrelated and thus this division is merely an arbitrary conceptual aid. The first line of mucosal defense limits

the amount of antigen coming into contact with the epithelial surface and consists of luminal factors including gastric acid and digestive enzymes, nonspecific antimicrobial substances and IgA antibodies secreted into the lumen, and an intestinal mucus layer coating the mucosal surface. The acidic environment of the stomach and upper duodenum is lethal to many ingested pathogenic bacteria and may affect proteolysis of antigens by digestive enzymes and hence reduce availability of intact antigens for uptake. In addition, intestinal epithelial cells have interestingly been observed to create an acidic microclimate on their apical surface.¹⁰ Digestive enzymes by definition cleave intraluminal antigens and as a consequence have a profound impact on their immunogenic properties, thus reducing the antigenic load. Intestinal immune cells secrete a number of nonspecific humoral defense factors including lysozyme, lactoferrin, peroxidase, defensins, and trefoil peptides which exhibit a variety of antimicrobial activities.

Secretory (s)IgA is a dimeric mucosal antibody produced by plasma cells in the lamina propria and transported across the epithelial layer bound to a polymeric immunoglobulin receptor. Dimeric IgA is secreted in the gut lumen bound to a cleaved portion of the polymeric immunoglobulin receptor known as secretory component to protect against proteolysis. sIgA antibodies bind to and coat pathogens, toxins, commensal microbes, and also dietary antigens, thereby reducing their capacity to bind to the gut epithelium. sIgA has been considered an anti-inflammatory antibody, as binding by sIgA does not typically result in inflammatory immune responsiveness. As alluded to above, a compromised gut barrier function may increase the risk of sensitization to dietary antigens and prior exposure to dietary antigens has been reported to reduce uptake of intact food proteins as a result of

induction of sIgA.¹¹ As an example of this function, IgA-deficient individuals have a heightened frequency of food allergy.¹² The role of sIgA in immune responses toward dietary antigens remains elusive, however, as healthy individuals have little or no food-specific intestinal IgA antibodies.¹³

The epithelial surface of the gastrointestinal tract is lined with a mucus coat composed of a mixture of mucin glycoproteins which vary greatly in both molecular weight (ranging from one to several million daltons) and carbohydrate side chains attached to the mucin protein skeleton.¹⁴ The thick mucus coat of the stomach protects the underlying mucosa from gastric acid damage. In addition, mucus viscosity and sIgA antibodies within the mucus layer trap particles including toxins and pathogenic bacteria. Intestinal epithelial cells, particularly goblet cells, continuously produce mucin molecules and the entrapped particles are expelled by gut peristalsis with shed mucus.

The Intestinal Epithelium and Innate Immune Responses.

Epithelial cells lining the surface of the body form a protective physical barrier against the external environment. The intestinal epithelium, however, must simultaneously be able to selectively absorb nutrients and therefore the epithelium in the alimentary tract (with the exception of the oral cavity, esophagus, and rectum) consists of a single layer of cells. The majority of the cells in the epithelial monolayer consist of polarized absorptive cells referred to as enterocytes. The apical surface of enterocytes is covered with negatively charged protrusions or microvilli, 100 μm in diameter. There are approximately 40 microvilli per 5 μm in the intestinal epithelium of children.¹⁵ The lipid bilayer microvillus membrane and the dense microvilli with the overlying glycocalyx formed by membrane-bound glycoproteins constitute a significant barrier to antigen entry. Paracellular antigen penetration is prevented by tight junctions between apical surfaces of enterocytes. The permeability of tight junctions may be modified by cytokines, such as tumor necrosis factor (TNF)- α and transforming growth factor (TGF)- β , or substances secreted by pathogens.^{16,17}

Intestinal epithelial cells express a number of PRRs to recognize microorganism-associated molecular patterns (MAMPs). MAMPs are present on both commensal and pathogenic microbes and required for their survival and therefore evolutionarily highly conserved.¹⁸ These conserved microbial structures include unmethylated CpG motifs characteristic of bacterial DNA, virus-derived single-stranded RNA, bacterial flagellin, and bacterial cell wall compounds such as lipoteichoic acid, muramyl dipeptide, peptidoglycan, and lipopolysaccharide.^{4,18,19} In contrast, with the exception of nucleic acids, MAMPs are not expressed by the host. Intestinal PRRs and their ligands are presented in Table 2.

The most extensively investigated epithelial PRRs to date are TLRs. Thus far, 10 individual TLRs (TLR1-10) have been identified in humans

Table 1 Components of the Intestinal Mucosal Barrier

1. External barrier
 - Gastric acid
 - Digestive enzymes
 - Nonspecific antimicrobial factors (eg, lysozyme, lactoferrin, peroxidase, defensins)
 - Secretory IgA
 - Intestinal mucus
 - Peristalsis
2. Epithelial layer
 - Microvilli
 - Plasma membrane
 - Tight junctions
 - Innate immune receptors
 - Intraepithelial immune cells
3. Intestinal lymphoid tissue
 - Uptake and presentation of antigens
 - Induction of secretory IgA production
 - Local and systemic inflammatory responses
 - Maintenance of tolerance
4. Indigenous intestinal microbiota
 - Degradation of luminal antigens
 - Inhibition of colonization and adherence of pathogens
 - Stimulation of the intestinal immune system

Table 2 Intestinal Pathogen Recognition Receptors and Their Known Ligands

Receptor	Ligand
TLR1	Lipopeptides
TLR2	Lipopeptides and lipoteichoic acid of gram-positive bacteria
TLR3	Viral dsRNA, viral sense ssRNA
TLR4	LPS
⁺ CD14	
⁺ MD-2	
TLR5	Bacterial flagellin
TLR6	Lipoteichoic acid
TLR8	Viral antisense ssRNA
TLR9	CpG DNA
NOD1	Diaminopimelic acid of peptidoglycan
NOD2	Muramyl dipeptide

CD-cluster of differentiation; CpGDNA- unmethylated cytosine-guanine-rich deoxyribonucleic acid; dsRNA-double-stranded ribonucleic acid; LPS-lipopolysaccharide; MD-2-myeloid differentiation protein-2; MyD88-myeloid differentiation primary-response protein 88; NOD-nucleotide-binding oligomerization domain; ssRNA-single-stranded ribonucleic acid; TLR-Toll-like receptor.

and TLR2-6 and TLR9 have been detected on human intestinal epithelial cells.^{18,20-23} In addition, TLR1-6, TLR8, and TLR9 messenger RNAs are expressed by human intestinal epithelial cells.²⁴ TLRs are also present on intestinal macrophages and dendritic cells (DCs).^{23,25} Structurally, TLRs are transmembrane proteins with a short transmembrane region.^{18,26} The extracellular domain of TLRs includes a leucine-rich repeat region with divergent ligand-binding properties. The intracellular domain contains a structure common to TLR and the interleukin (IL)-1 receptor family referred to as the Toll/interleukin 1 receptor (TIR). The TIR domain is essential for initiation of TLR signaling, which may lead to activation of a number of transcription factors and, consequently, the induction of a variety of immune response genes. The type of immune activation induced via different TLRs is dependent on the cofactors and adaptor proteins involved in signal transduction. Antigen binding by all known TLRs is capable of activating the myeloid differentiation primary-response protein 88 (MyD88)-dependent pathway, which involves activation of members of interleukin 1 receptor-associated kinase family and leads to expression of inflammatory genes through activation of a number of transcription factors including nuclear factor κ B (NF- κ B) and activator protein 1.²⁶ In addition to MyD88 activation, at least four alternate TLR signaling pathways involving the adaptor proteins Mal/TIRAP, TRIF/TICAM-1, TRAM/Tirp/TICAM-2, or SARM have been identified.²⁷ Recognition of pathogens by TLRs thus rapidly initiates protective immune responses. Interestingly, however, inflammatory immune responsiveness against harmless indigenous microbes binding to TLRs appears to be prevented by sophisticated mechanisms involving the IL-1 receptor associated kinase activation suppressor Toll-interacting protein and NF- κ B agonist peroxisome proliferators-activated receptor

γ (PPAR γ).²⁴ Other inhibitors of TLR signal transduction include single immunoglobulin IL-1R-related protein, suppressor of cytokine signaling, the zinc finger protein A20, and the cytokines TGF- β and IL-10.²⁸

Two PRRs in the NOD family, NOD1 and NOD2 [also known as caspase activation and recruitment domain (CARD)4 and CARD15, respectively], of cytosolic receptors have recently been investigated with regard to their role in regulation of intestinal immunology. Both NOD1 and NOD2 bind to bacterial cell wall structures. The specific ligands are γ -D-glutamyl-mesodiamino-pimelic acid of gram-negative bacteria for NOD1 and muramyl dipeptide for NOD2.^{29,30} Ligand binding to NOD1 or NOD2 leads to NF- κ B activation via the Rip2/RICK/CARDIAK serine-threonine kinase but the details of the signaling pathway still remain elusive.³¹

Indigenous Intestinal Microbiota. The human gastrointestinal tract harbors an enormous quantity of indigenous microbes. The number of prokaryotic cells in the intestine has been estimated to be 10^{14} , the vast majority of which are found in the colon. These microbes belong to more than 500 culturable species and through the emergence of culture-independent molecular identification techniques the number of microbial species constituting the gut microbiota has been suggested to exceed 1,000. This complex ecosystem of microbes is referred to as the intestinal microbiota. Colonization of a sterile gastrointestinal tract commences at birth and proceeds in a stepwise and systematic fashion. Maternal intestinal and vaginal microbiota provide the first bacterial inoculum to the newborn and hence facultative gram-positive cocci (staphylococci, streptococci, and enterococci) and enterobacteria are the first bacteria to colonize the intestine. Consequently, gut microbiota composition in infants born by caesarian section differs significantly from that of vaginally born infants.³² This has been suggested to partially explain the heightened risk of atopic disease and sensitization to dietary antigens in infants born by caesarian section.³³ Early nutrition has a profound impact on the composition of the infant's gut microbiota. In particular, breast-feeding promotes the growth of bifidobacteria, which become the predominant microbiota species in breast-fed infants.³⁴ After weaning the gut microbiota composition of infants resembles that of adults.

Intestinal microbes have been shown to be necessary for normal morphological and immunological maturation of the gut in experimental studies conducted using germ-free animals.³⁵ Germ-free mice exhibit impaired sIgA production.³⁶ Moreover, specific strains of intestinal microbiota have been shown to modulate the expression of host genes involved in a variety of intestinal functions including nutrient absorption, metabolism, mucosal barrier function, and intestinal maturation.³⁷ It has recently been reported that defective immune maturation in germ-free mice could be corrected by colonization with *Bacteroides fragilis*, a member of murine indigenous intestinal microbiota.³⁸

As discussed above, PRRs of the intestinal immune system recognize MAMPs present on both pathogens and indigenous intestinal

microbes. Interestingly, however, pathogen recognition by TLRs leads to inflammatory responsiveness mediated by the NF- κ B pathway, whereas nonpathogenic intestinal microbes have been observed to suppress the same pathway.^{19,39} There are also data indicating that intestinal epithelial cells cultured in vitro are unresponsive to TLR ligands on indigenous intestinal microbes.²² Monocolonization of germ-free rats with the commensal *Bifidobacterium lactis* has been shown to transiently activate the NF- κ B pathway via TLR2 signaling but this activation does not result in tissue damage.⁴⁰ Moreover, recognition of commensal bacteria through TLRs appears to be necessary for protection against epithelial injury, as mice deficient in TLR2, TLR4, or MyD88, a downstream TLR signaling molecule, exhibit increased morbidity and mortality from chemically induced colitis.⁴¹ It has recently been shown that while intestinal macrophages rapidly kill commensal bacteria that penetrate the surface epithelial layer, intestinal DC may retain live bacteria intracellularly and induce protective sIgA production in the mesenteric lymph nodes without systemic immune activation.⁴²

Indigenous microbes contribute to intestinal barrier function by degrading intraluminal antigens and by inhibiting adherence and colonization by pathogenic microbes. Antigen transport across the intestinal epithelium is increased in the absence of intestinal microbes in experimental animals. The immunomodulatory consequences of luminal antigen degradation by microbial proteases are demonstrated by in vitro experiments in which lymphocyte proliferation and production of IL-4 were suppressed by cow's milk casein hydrolyzed with enzymes derived from lactobacilli.^{43,44}

Antigen Presentation and Induction of Adaptive Immunity in the Gut-Associated Lymphoid Tissue. Despite the effects of degradation by digestive enzymes of the host or indigenous microbes and coating with antigen-specific sIgA antibodies in the gut lumen discussed above, a nonnegligible amount of immunologically intact macromolecules reaches the intestinal surface. Intestinal adaptive immune responses against ingested antigens are orchestrated by T lymphocytes of gut-associated lymphoid tissue (GALT). Instead of being an anatomically well-defined lymphoid organ, GALT is composed of a complex network of immune cells distributed within the intestine as isolated intraepithelial and lamina propria lymphocytes or organized lymphoid structures such as Peyer's patches (PPs) and lymphoid follicles in the small intestine and colon. PPs located in the small intestinal submucosa are thought to be the principal induction site of intestinal adaptive immunity. The PP is covered with specialized epithelium referred to as follicle-associated epithelium characterized by the presence of microfold (M) cells which lack protective microvilli and thick mucus.⁴⁵ M cells are so named because of their basolateral surface, which has pockets enfolding lymphocytes, DCs, and macrophages. It is believed that antigens are not degraded by M cells but delivered intact to be

processed by the underlying APCs. Recently, signaling through TLRs has been shown to regulate the functions of the follicle-associated epithelium, as TLR2 activation was observed to enhance trans-epithelial transport of antigens by M cells.⁴⁶

Intestinal DCs are professional APCs which express microbial peptides bound to class II major histocompatibility (MHC) molecules on their surface and activate T-cell responses after having migrated to mesenteric lymph nodes. DCs residing in M-cell pockets ingest and sample antigens passing through M cells. In addition, there are data to suggest that DCs may monitor the intestinal content by extending dendrites into the gut lumen after opening tight junctions.⁴⁷ Activation, maturation, and trafficking of intestinal DCs are dependent on microbial stimulation through TLRs and DCs, in turn, play a pivotal role in determining the type of adaptive immune responses to be launched (Figure 3).⁴⁸

Based on cytokine production patterns, adaptive immune responses may be divided into cross-regulatory Th1 and Th2 subsets. Th1 cells are characterized by secretion of IFN- γ , IL-2, and TNF- α , with little or no IL-4, IL-5, IL-9, or IL-13, and orchestrate immune defense against intracellular pathogens. Th2 cells, on the other hand, secrete IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, but not IFN- γ or TNF- α , and are implicated in humoral responses, especially those of the IgE class, and are involved in defense against helminthic infections. Both Th1- and Th2-type responses have been implicated in the pathogenesis of human disease.⁴⁹ Inflammatory immune reactions driven by Th1-type responses are encountered in many autoimmune states whereas Th2-type responsiveness is central in the pathogenesis of allergic disorders. In particular, Th2-skewed immune responsiveness has been detected in individuals with food allergy.⁵⁰

There are limited data regarding the type of T-cell responsiveness preferentially elicited

toward dietary antigens in the intestine. An early study conducted using a mouse model suggested that PP T-cell responses are preferentially of the Th2 type with pronounced IL-5 production.⁵¹ Subsequent studies on human subjects, however, have shown that human PP T cells spontaneously produce considerable amounts of the Th1 cytokine IFN- γ .⁵² Moreover, PP T cells have been reported to respond to stimulation with cow's milk antigens by production of IL-12 and expansion of CD4⁺CD25⁺ T cells.⁵³ Th1 and Th2 cells develop from naïve CD4⁺ T cells which become polarized under the influence of genetic and microenvironmental factors and there is a cross-regulatory balance early in adaptive responses with Th1 cytokines inhibiting Th2 responses and vice versa.⁵⁴ IL-12 and IFN- γ thus promote further Th1 polarization and it is therefore likely that human PP T-cell responses toward dietary antigens in nonallergic individuals are preferentially of the Th1 type, even though food-antigen-specific effector T cells are usually not found in the intestinal mucosa.⁵⁰ Food proteins per se appear to affect the maturation of the intestinal immune system since animals receiving a protein-free diet, albeit not malnourished, display defective Th1 responsiveness and limited production of IgA and IgG antibodies.⁵⁵

Establishment and Maintenance of Immune Tolerance to Dietary Antigens

Specific immune functions have developed to prevent inappropriate and potentially detrimental inflammatory reactions against food antigens. These functions as a whole and the resulting apparent nonresponsiveness are referred to as oral tolerance. An intricate network of regulatory immune responses has developed in the gut to orchestrate the establishment and maintenance of oral tolerance which is induced by small amounts of antigens crossing the mucosal surface in

immunologically intact form. The active nature of immune tolerance is underscored in a definition suggested by Weiner according to which tolerance is defined as "any mechanism by which a potentially injurious immune response is prevented, suppressed, or shifted to a noninjurious class of immune response."⁵⁶

In experimental studies, oral tolerance has successfully been induced against antigens as diverse as heavy metals, heterologous red blood cells, allogeneic leukocytes, extracts of pollen and other aeroallergens, inactivated viruses and bacteria, and a large variety of proteins.⁵⁷ In addition, the host is tolerant to its indigenous microbiota. Abrogation of oral tolerance to dietary antigens results in food allergy whereas inappropriate inflammatory immune responsiveness against indigenous intestinal microbiota has been described in IBD.⁵⁸ Several studies indicate that oral tolerance cannot be induced in germ-free animals.⁵⁹ Sudo and colleagues have elegantly demonstrated that mice raised in germ-free conditions exhibit impaired development of the intestinal immune system and defective oral tolerance formation with an excessive Th2-type immune responsiveness.⁶⁰ Reconstitution of the intestinal microbiota with bifidobacteria in these animals restored the ability to establish oral tolerance in the neonatal period but not at a later age. In a similar fashion, oral administration of lipopolysaccharide has been reported to induce tolerance in germ-free mice.⁶¹ Recent advances in understanding innate immunity suggest that the modulation of tolerance might be mediated via DCs or directly through TLRs on regulatory CD4⁺CD25⁺ T cells.^{62,63} In accord with this observation, indigenous microbes have been reported to be necessary for the induction of regulatory T cells (T_{reg}s).⁶⁴

T-Cell Deletion and Anergy. Ingestion of large doses of antigen may lead to inactivation of antigen-specific T cells. There are experimental data to suggest that feeding large quantities of antigen lead to clonal deletion of T cells but the significance of this phenomenon in oral tolerance is not clear.⁶⁵ T-cell anergy refers to a state of antigen-specific hyporesponsiveness characterized by an inability to produce IL-2 resulting from priming with the same antigen. Stimulation of the T-cell receptor in the absence of proinflammatory costimulatory molecules on the APC or costimulatory signaling through the cytotoxic T-lymphocyte-associated protein (CTLA)-4 on T cells leads to T-cell anergy.⁶⁶ Anergic T cells may in some instances act as T_{reg}s and therefore T-cell anergy and T_{reg} function may be regarded as two aspects of the same cell rather than distinct mechanisms of tolerance.^{57,59}

Active Immunosuppression by Regulatory T cells. Distinct regulatory mechanisms mediated by regulatory T-cell subsets including gut-derived Th3 and T regulatory 1 cells and thymus-derived or peripherally induced CD4⁺CD25⁺ T cells, modulate adaptive Th1 and Th2 responses.⁶⁷ Th3 and T regulatory 1 cells are thought to exert their effects through production of the cytokines TGF- β and IL-10, respectively, while CD4⁺CD25⁺ T cells mainly act through contact-dependent mechanisms involving CTLA-4 and membrane-bound TGF- β .⁶⁷⁻⁶⁹

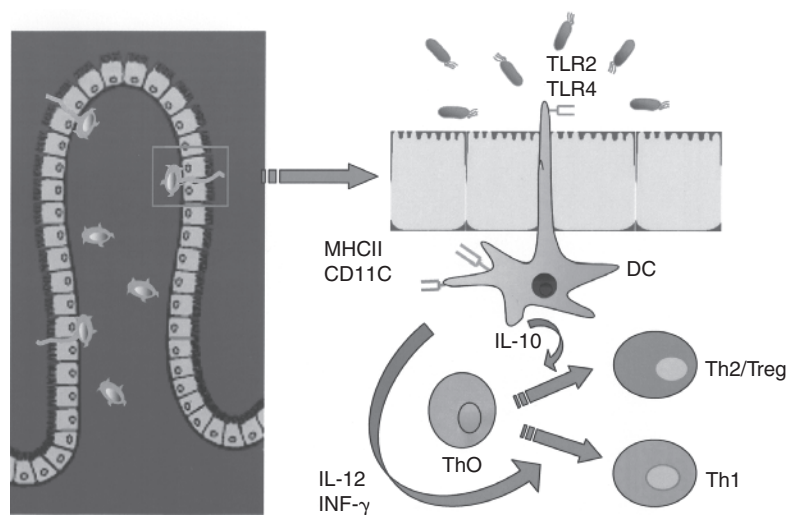


Figure 3 Recognition of luminal antigens by dendritic cells (DCs) and induction of adaptive immune responses. Intestinal DCs expressing CD11c sample luminal contents by extending dendrites expressing Toll-like receptors through tight junctions and then present microbial peptides bound to class II major histocompatibility molecules to naïve T cells. This antigen presentation by DCs plays a decisive role in determining the type of adaptive immune responses to be launched. Intestinal DCs producing IL-10 promote the development of T helper (Th)2 cells or regulatory T cells, whereas Th1 polarization develops in the presence of IL-12 and IFN- γ . CD = cluster of differentiation; IFN- γ = interferon- γ ; IL = interleukin.

However, both the nomenclature and the functional or developmental interrelations of these regulatory T-cell populations are still somewhat unclear.

Oral administration of antigen leads to the generation of TGF- β -secreting Th3 cells in both animal models and human subjects.^{70,71} TGF- β induces the development of CD4⁺CD25⁺ T cells through the transcription factor FOXP3.⁷² Antigen feeding has been shown to lead to the induction of CD4⁺CD25⁺ T cells in the GALT and the number of CD4⁺CD25⁺ T cells expressing FOXP3 and CTLA-4 is increased in PPs after oral exposure to antigen.^{73–75} There are a number of studies indicating that T_{reg}s are implicated in clinical food allergy and development of tolerance in human subjects. Infantile food allergy may be considered a manifestation of a primary failure to establish oral tolerance. A defect in regulatory adaptive immune responses in food allergy is suggested by a report according to which cow's milk-specific T-cell clones isolated from peripheral blood of children with cow's milk allergy (CMA) exhibit diminished IL-10 production compared to those from tolerant children.⁷⁶ In addition, the number of TGF- β -producing T cells has been reported to be reduced in the intestinal epithelium and lamina propria of children with food allergy.⁷⁷ Interestingly, the development of tolerance in children with CMA is associated with the appearance of circulating allergen-responsive CD4⁺CD25⁺ T cells.⁷⁸ Nonetheless, CD4⁺CD25⁺ T cells appear to be present and functional in adult patients with CMA.⁷⁹

IMMUNOMODULATORY FACTORS IN NUTRITION

Nutrition and nutrients have diverse effects on host immunophysiology. During the first months of life, when the immune system still lacks mature competence and oral tolerance and the intestinal microbiota are first established, breast milk provides the infant with both passive immunoprotection in the form of soluble antimicrobial molecules and maturational signals including growth factors and cytokines. Lack of proper nutrition affects host immunophysiology and these effects contribute to the morbidity and mortality caused by malnutrition. A number of nutrients, including vitamins, iron, zinc, and other trace elements, glutamine and other amino acids, and certain lipids influence immune functions by mechanisms beyond correction of dietary deficiencies.

Breast Milk

Breast milk provides the newborn infant with optimal nutrition for growth and development and immunoprotection during a critical period when the neonatal immune defenses are still immature. Infectious disease is a major threat to the neonate and the leading cause of infant mortality in many areas of the world. It is well established that breast-feeding protects the infant from infectious disease. This protective effect is mediated by a number of passive antimicrobial factors, including lysozyme, lactoferrin, peroxidase, defensins,

cathelicidin, sIgA, TLRs with soluble CD14, and oligosaccharides which are discussed in detail in Chapter 31, "Protective Properties of Human Milk," of this volume. The protection provided by breast milk is crucial in developing countries, where breast-feeding also reduces the infant's need to consume potentially contaminated water. In addition to passive immunoprotection, breast milk contains factors which contribute to immune maturation by stimulating the intestinal immune system either directly or via modulating the indigenous intestinal microbiota.

The role of breast milk in reducing the risk of NEC in premature neonates is well established.⁸⁰ This effect may partially be mediated by TGF- β , which is present in colostrum in significant amounts. TGF- β has been shown to attenuate intestinal epithelial cell inflammatory responses associated with the development of NEC.⁸¹ Increased gut permeability during the first days of life may increase the risk of NEC. Breast-feeding has been shown to improve the intestinal barrier in the neonatal period as assessed by the absorption of macromolecules in a rabbit model and TGF- β is known to enhance intestinal repair.^{82,83}

The long-term immunological consequences of breast-feeding have most thoroughly been investigated in the context of immunologically mediated diseases such as asthma and atopic disease. The epidemiological data on the subject are somewhat discrepant, which may be explained by differences in breast milk composition between individuals which in turn may partially result from hereditary factors predisposing to atopic disease. Nonetheless, a report from a prospective birth cohort study of more than 2,000 children in Australia indicates that the duration of exclusive breast-feeding is associated with a reduced risk of asthma and atopic sensitization.⁸⁴ The exact mechanisms through which breast milk enhances immune maturation and thereby protects from the development of immunopathology in infants are not well characterized. It has been demonstrated, however, that low breast milk concentrations of TGF- β or n-3 fatty acids are associated with increased risk of subsequent development of atopic disease.^{85,86}

Aberrant Nutritional States and Immune Function

Malnutrition. Malnutrition is still a major burden to child health in large areas of the world. Insufficient intake of energy and protein is often accompanied by deficiency of a variety of vitamins, minerals, and other factors related to host immunophysiology. Indeed, malnutrition is the most common cause of immunodeficiency in children. Long-term deficiency of energy, protein, vitamins, and minerals results in marasmus or nonedematous malnutrition whereas more acute malnutrition, induced by, for example, infection, often leads to an edematous state of malnutrition referred to as kwashiorkor. A combination of the aforementioned states may also be encountered.

The immunological consequences of malnutrition have been investigated in experimental animal models. In addition to reducing growth,

energy malnutrition has been observed to result in aberrant intestinal mucin production and severe protein deficiency leads to a drastic decline in intestinal sIgA production in rats.^{87,88} According to recent reports, acute malnutrition increases serum concentrations of the regulatory cytokines IL-10 and TGF- β 1 as well as IgE and IgG1 antibodies in mice.^{89,90}

Children with severe protein and energy malnutrition exhibit pathological small intestinal architecture characterized by villous atrophy.^{91,92} Importantly, increased intestinal permeability as assessed using the lactulose-mannose test has been reported even in malnourished individuals without histological intestinal abnormality.⁹³ The significance of this phenomenon remains to be elucidated but it may be hypothesized that abrogation of the gut barrier may provide a route of entry for pathogens and thus contribute to the heightened risk of infectious disease in malnourished children. The effects of malnutrition on host defense are discussed in detail in Chapter 23, "Malnutrition and Host Defenses." Infection and malnutrition may have overlapping effects on gut immune function in the same individual. However, malnourished children suffering from infections have been observed to have decreased numbers of circulating CD45⁺RO⁺ memory T cells as compared to well-nourished children with similar infections.⁹⁴

In a study conducted by Campbell and colleagues, small bowel biopsies were obtained from 38 Gambian children in different nutritional states and compared to biopsies from 19 children from Britain.⁹⁵ Interestingly, all Gambian children regardless of nutritional status exhibited signs of chronic cell-mediated enteropathy with increased intestinal permeability, villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis characterized by infiltration by CD25⁺ cells. Malnutrition was associated with reduced numbers of intestinal T and B cells. Moreover, the expression of TGF- β in the intestinal mucosa decreased progressively with a worsening nutritional state suggesting impaired immunoregulation as a result of protein-energy malnutrition.

Total Parenteral Nutrition. Parenteral nutrition is a crucial element in the care of severely ill neonates, infants, and children, as discussed in detail in Chapter 69, "Parenteral Nutrition," but it is also associated with increased risk of infectious complications. While risks related to central venous catheters are likely to explain increased morbidity to some extent, it is also well established that total parenteral nutrition (TPN) may lead to atrophy of intestinal villi and lamina propria, epithelial cell apoptosis, and increased intestinal permeability.^{96,97} In addition, a significant reduction in the number of lymphocytes in PPs and the lamina propria as well as intraepithelial lymphocytes is seen as a result of TPN in mice.⁹⁸ Both B- and T-cell numbers and lamina propria CD4⁺ T cells in particular appear to be affected by the lack of enteral nutrition.^{99,100} There are data to suggest that the mucosal atrophy resulting from TPN may be reduced by using TPN supplemented with short-chain fatty acids (SCFAs) or glutamine.^{101,102} The importance

of enteral nutrition or antigenic stimulation to both intestinal microbiota and gut immunophysiology is demonstrated by studies indicating that TPN results in a reduction of both aerobic and anaerobic indigenous bacteria and decreased intestinal concentrations of IgA, IL-4, and IL-10.^{100,103,104}

Selected Nutrients

Trace Elements. Zinc is a micronutrient essential to intestinal health. Children suffering from acrodermatitis enteropathica, a condition characterized by severe zinc deficiency resulting from a genetic defect in intestinal zinc transport, manifest with chronic diarrhea and dermatitis.¹⁰⁵ Dietary zinc deficiency leads to increased epithelial permeability due to decreased tight junction integrity in the rat intestine.¹⁰⁶ In humans, zinc deficiency has been reported to be associated with increased susceptibility to infections, which suggests a role for zinc in immunophysiology.¹⁰⁷ Indeed, zinc has been observed to be involved in a number of immune functions including T-cell responses and production of antibodies and cytokines.^{108,109} Zinc deficiency leads to decreased NF- κ B activity and loss of precursor T and B cells in the bone marrow which in turn results in lymphopenia and thymic atrophy.^{109,110}

Based on the role of zinc in immunophysiology, it has been hypothesized that zinc supplementation may improve host immunity and that these beneficial effects might extend beyond correcting deficient dietary zinc intake. Zinc supplementation has been shown to accelerate mucosal regeneration, increase intestinal brush border enzymes and secretory antibody concentrations, and enhance cellular immunity.¹¹¹ Nonetheless, the role of zinc supplementation in clinical practice has not yet been established. In the developing world, zinc supplementation during diarrheal disease has been reported to reduce the duration and severity of diarrhea and enhance catch-up growth.¹¹² Contrary to previous studies, however, a recent report from a trial conducted in India failed to show improved recovery from pneumonia by zinc supplementation in hospitalized children less than 2 years of age.¹¹³ Zinc supplementation did not affect growth, development, or infection risk in term breast-fed infants in a study carried out in the United States.¹¹⁴

Dietary deficiencies in copper or iron are associated with increased susceptibility to infections.^{115,116} Copper deficiency is associated with impaired cell-mediated immunity characterized by defective macrophage activation and cytokine production.¹¹⁵ Iron deficiency on the other hand has been observed to impair T-cell function and IL-1 production.^{117,118} Dietary copper deficiency in infants and children is rare. In contrast, deficiency in iron is not uncommon and it is therefore noteworthy that excessive iron intake, which may result from inappropriate supplementation, may also increase the risk of infectious disease.¹¹⁹

Glutamine and Arginine. The nonessential amino acid glutamine influences host immune functions and is critical during catabolic stress.¹²⁰ Glutamine is an important nutrient to both enterocytes and immune cells and depletion of

glutamine leads to intestinal mucosal damage and atrophy in experimental animals.¹²¹ In human intestinal tissue cultures, glutamine has been reported to reduce the production of the inflammatory cytokines IL-6 and IL-8.¹²² There is data to suggest that this reduction in intestinal inflammatory responsiveness is mediated by inhibition of I κ B α ubiquitination.¹²³ As alluded to above, glutamine supplementation has been reported to reverse a number of detrimental consequences of TPN: Experimental animals receiving glutamine-supplemented TPN exhibit improved integrity of the intestinal barrier as well as less impaired IgA and cytokine responses compared to animals receiving conventional TPN.^{124–126}

In clinical trials, glutamine-supplemented TPN has been demonstrated to decrease the risk of infectious complications in critically ill patients and after bone-marrow transplantation.^{127,128} Two randomized trials with relatively small numbers of subjects have suggested that enteral glutamine supplementation might reduce the risk of septicemia in very low birth weight neonates.^{129,130} However, a multicenter trial with 649 very low birth weight neonates found no differences in the occurrence of verified or suspected sepsis in infants receiving enteral glutamine or placebo, nor did parenteral glutamine supplementation reduce mortality in a study of more than 1,400 extremely low birth weight infants.^{131,132}

Arginine is a nonessential amino acid which is known to modulate immunophysiology by enhancing proliferation and cytotoxicity of lymphocytes.¹³³ In an animal model of septic peritonitis, enteral but not parenteral arginine supplementation prior to the onset of disease resulted in significantly improved intestinal immune responses by enhancing IgA and IL-10 secretion.¹³⁴ The clinical effects of arginine supplementation are as of present difficult to assess because most trials with nutritional intervention include arginine in combination with other immunomodulatory nutrients. Early enteral nutrition with arginine, fiber, and antioxidant supplementation reduced the risk of catheter-related sepsis but did not reduce mortality in critically ill patients in a Spanish multicenter study.¹³⁵ These data are consistent with a meta-analysis of 22 clinical trials, which concluded that commercial nutrition products with high arginine contents are associated with reduced infectious morbidity but do not affect overall mortality.¹³⁶

Vitamins. Vitamin A is required for optimal mucosal immune function. Vitamin A deficiency has been shown to lead to compromised gut barrier function with impaired sIgA and mucus production and increased bacterial translocation.^{137–139} The reduced IgA production may in part be explained by data according to which the number of IL-4 and IL-5 producing T cells which induce humoral responses is reduced in the intestine of vitamin A-deficient mice.^{140,141} Correcting the deficiency with vitamin A supplementation has been shown to restore both IgA concentrations and mucosal integrity.^{141,142}

Children deficient in vitamin A exhibit heightened risk for infections in the gastrointestinal and respiratory tracts. An impressive 20 to 50%

reduction in childhood mortality has been reported from developing countries after implementation of large-scale vitamin A supplementation programs.¹⁴³ Nonetheless, recent meta-analyses of clinical trials assessing the effects of vitamin A supplementation on infectious disease morbidity or mortality have yielded somewhat contradictory results: One meta-analysis of randomized clinical trials concluded that vitamin A supplementation resulted in a 30% reduction in infant and child mortality whereas three other meta-analyses have failed to find significant reduction in either morbidity or mortality in diarrheal disease or pneumonia.^{144–147} There are no convincing data to suggest that vitamin A supplementation beyond correcting a state of deficiency is associated with beneficial enhancement of immune functions.

In addition to its role in maintaining calcium balance, vitamin D has a number of immune regulatory properties. The active form of vitamin D, 1,25-dihydroxyvitamin D₃, modulates Th1 and Th2 cell responses and there are experimental data which indicate that suppression of Th1 responsiveness by vitamin D may ameliorate Th1-mediated autoimmune disease.¹⁴⁸ Vitamin D deficiency is associated with increased susceptibility to respiratory tract infections in children and it also appears to be linked to the development of immune-mediated diseases such as IBD.^{148,149} However, especially bearing in mind the potential toxicity of vitamin D, there are no data to imply benefit of vitamin D supplementation beyond correcting dietary deficiency.

Vitamin E (α -tocopherol) is a fat-soluble vitamin which scavenges free oxygen radicals. Oral supplementation of a water-soluble vitamin E derivative has been shown to limit chemically induced colitis in rats.¹⁵⁰ In addition, dietary vitamin E has immunomodulatory properties beyond its antioxidant function including effects on lymphocytes and antibody and cytokine production.¹⁵¹ Vitamin E has been demonstrated to suppress Th2 cytokine responses and IgE production in a murine asthma model.¹⁵² However, supplementation with vitamin E has not been of benefit to patients with allergic rhinitis or asthma in clinical trials.^{153,154}

Vitamin C (ascorbic acid) has antioxidant and immunomodulatory effects. Consequently, vitamin C supplementation is widely used to prevent and treat viral infections of the respiratory tract. According to a meta-analysis of clinical trials, however, vitamin C supplementation does not reduce the incidence of common colds in the general population but may reduce the risk of colds in people exposed to heavy physical or cold stress.¹⁵⁵ In addition, a small but consistent beneficial effect on the duration and severity of cold episodes was detectable in individuals using regular vitamin C prophylaxis but the clinical significance of this effect is questionable. Currently existing data do not support the use of vitamin C supplements to enhance immune functions in non-vitamin C-deficient individuals.

Fatty Acids. Fatty acids have a wide array of immunomodulatory effects. Fatty acids from diet

and cellular lipids are precursors for a number of immune molecules referred to as eicosanoids, which include prostaglandins, thromboxanes, and leukotrienes.¹⁵⁶ In addition, fatty acids and their metabolites can influence immune cell gene expression by activating PPAR nuclear transcription factors.¹⁵⁷ Polyunsaturated fatty acids (PUFAs) and SCFAs have been most extensively studied with regard to their effects on host immunophysiology.

Both n-3 and n-6 PUFAs bind to PPARs.¹⁵⁸ A diet rich in n-6 PUFA has been reported to increase blood mononuclear cell mitogenic responses, as well as IL-1 β and TNF production in vitro, while n-3 PUFA diet was associated with a decrease in the production of these inflammatory cytokines.¹⁵⁹ However, subsequent studies have provided discrepant data regarding the anti-inflammatory effects of n-3 PUFA and the issue is in need of further clarification. In rats, dietary linoleic acid, eicosapentaenoic acid, and docosahexaenoic acid (DHA) inhibit lymphocyte proliferation.^{160,161} Eicosapentaenoic acid has also been reported to suppress APC function by reducing the expression of MHC II molecules.¹⁶² SCFAs, on the other hand, have been associated with increased intestinal IL-1 β and IL-6 responses in rats receiving TPN.¹⁶³ Whether dietary intake of PUFAs above current recommendations offers additional health benefits remains to be elucidated.

FUTURE DIRECTIONS: DIETARY INTERVENTIONS AIMED TO INFLUENCE INTESTINAL IMMUNOPHYSIOLOGY

Probiotics

Probiotics are live microorganisms, which when administered in adequate amounts confer a health benefit to the host.¹⁶⁴ Probiotic effects include degradation of dietary antigens, promotion of mucosal barrier functions, inhibition of mucosal pathogen adherence, and interaction with the innate and adaptive immune systems of the host.¹⁶⁵ In addition to several identified potential targets for probiotic intervention, results from experimental studies and clinical trials suggest future indications for their therapeutic use.

Probiotics may be of benefit in reducing the risk and treatment of infectious disease as a result of the ability to promote mucosal barrier functions, inhibit mucosal pathogen adherence, and modulate host immunophysiology.¹⁶⁵ Two independent meta-analyses of double-blind, placebo-controlled clinical trials have concluded that probiotics are effective in treatment of acute infectious diarrhoea in infants and children.^{166,167} According to a recent meta-analysis, probiotics are also effective in reducing the risk of antibiotic-associated diarrhea in children.¹⁶⁸ In addition, there are clinical trials to suggest that *Lactobacillus* GG may also reduce the risk of acute infantile diarrhea in different settings, including nosocomial spread of infection and undernourished children in the developing world.^{169,170} The data on the effects of probiotics with regard to respiratory tract infections are more limited. The combination of the probiotics *B. lactis*

Bb-12 and *L. reuteri* was not effective in reducing the risk of respiratory tract infections during a 12-week period in a double-blind, placebo-controlled study of 201 infants.¹⁷¹ A modest reduction in the severity of respiratory tract infections and absence from daycare without significant reduction in the occurrence of infections has been reported using milk supplemented with *L. GG*.¹⁷²

Alterations in the intestinal microbiota composition in infancy precede the development of atopic disease suggesting a causal link between indigenous gut bacteria and the atopic phenotype.¹⁶⁵ Probiotic bacteria have a number of immunological effects which may contribute to reducing the risk and treatment of allergic disease. In experimental studies, probiotics have been observed to suppress IgE responses and Th2 cytokine production and induce T_{reg}s.^{165,173} Supplementation with *L. GG* during late pregnancy and lactation has been reported to induce anti-inflammatory mucosal immune responses indicated by an increase in the concentration of TGF- β 2 in breast milk.¹⁷⁴ *L. GG* and *B. lactis* Bb-12 supplementation commenced at the time of weaning has been reported to result in increased mucosal cow's milk-specific IgA production and increased serum concentrations of soluble CD14 in infants.³ Administration of *L. acidophilus*, however, had no effect on cytokine production patterns during the first 6 months of life in a recent study of 230 infants.¹⁷⁵

Several probiotic strains of lactobacilli have been reported to be effective in the treatment of atopic dermatitis in infants and children, and hypoallergic formula supplemented with *L. GG* has been shown to alleviate symptoms and the intestinal inflammation in infants with CMA and atopic dermatitis.¹⁶⁵ *L. GG* has also shown promising potential in reducing the risk of atopic dermatitis in infants at high hereditary risk for atopic disease. In a double-blind, placebo-controlled clinical trial of 159 pregnant women *L. GG* was administered 2 to 4 weeks prior to expected delivery and the probiotics were subsequently administered to either the breast-feeding mother or the infant until the age of 6 months.¹⁷⁶ Probiotic supplementation reduced the risk of atopic dermatitis in these high-risk infants from 46 to 23% during the first 2 years of life and this protective effect has subsequently been reported to extend to the age of 4 years.¹⁷⁷ These results need to be confirmed by larger studies before practical recommendations on the use of probiotics in prevention or treatment of atopic disease can be given.

The pathogenesis of NEC, albeit largely not yet clarified, involves increased gut permeability combined with enteral feeding and altered intestinal microbiota composition.^{178,179} Consequently, probiotics might be suggested to reduce the risk of developing NEC by promoting gut barrier function and inhibiting colonization by potentially pathogenic bacteria. A significant reduction in NEC occurrence has been reported from a neonatal intensive care unit in Colombia after supplementation of infants in the unit with the probiotics *L. acidophilus* and *B. infantis* was commenced.¹⁸⁰ Subsequently, two randomized, placebo-controlled

trials using different probiotic combinations have also found significantly reduced incidence of NEC compared to placebo whereas in one multicenter study *L. GG* offered no beneficial effect on the incidence of NEC, bacterial sepsis, or urinary tract infections.^{181–183}

IBD is characterized by mucosal inflammation as a result of detrimental immune reactivity toward indigenous intestinal microbes. Thus modulating the composition of either intestinal microbiota or mucosal immune responses by probiotics might provide a means to treat or even prevent IBD. *L. GG* has been shown to enhance intestinal IgA production in patients with Crohn's disease.¹⁸⁴ A probiotic strain of *E. coli* is effective in maintaining remission in patients with UC and the probiotic combination VSL#3 (containing *L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. longum*, *B. breve*, *B. infantis*, and *Streptococcus salivarius* subsp. *thermophilus*) has recently been shown to induce remission in mild-to-moderate UC.^{185,186} VSL#3 has also been demonstrated to be effective in prevention of acute pouchitis, a nonspecific inflammation of the ileal reservoir.¹⁸⁷ Furthermore, VSL#3 reduces the risk of recurrence of chronic pouchitis.^{188,189} These beneficial effects might be the result of induction of tolerogenic immune responses as administration of VSL#3 resulted in a significant increase in IL-10 in biopsies from patients with pouchitis.¹⁹⁰

Prebiotics

Nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of intestinal bacteria and thus promote host health are referred to as prebiotics.¹⁹¹ The most commonly used prebiotics include lactulose, inulin, and oligosaccharides such as fructo-oligosaccharides (FOSs) and galacto-oligosaccharides (GOSs). The effects of prebiotic oligosaccharides in promoting bifidobacteria in the intestinal microbiota of infants have been demonstrated in a number of double-blind, placebo-controlled trials.^{192–195} In addition to the stimulating effect on the growth of bifidobacteria, infant formula supplemented with GOS and FOS has been reported to result in intestinal microbial metabolic activity closer to that observed in breast-fed infants compared to infants fed unsupplemented formula.¹⁹⁵ Interestingly, prebiotics may also modulate nutrient intake as demonstrated by a study according to which administration of an inulin-type fructan to adolescents significantly increased calcium absorption and bone mineralization during pubertal growth.¹⁹⁶

There are limited published data regarding the immunological effects of prebiotics to date. No immunological effects, including no changes in serum immunoglobulin or sIgA concentrations or IL-4 and IFN- γ secretion by monocytes in vitro, were detected in a study assessing the effects of a mixture of prebiotic raffinose and rafterline in elderly people.¹⁹⁷ Infant cereal supplemented with FOS appeared to have no effect of *Haemophilus influenzae* B vaccination responses nor did it reduce the risk of diarrheal disease in a study

conducted in Peru.¹⁹⁸ Given the epidemiological link between intestinal microbiota composition and the development of atopic disease, prebiotics have been hypothesized to be of benefit in reducing the risk and treatment of atopic disorders. Recently surfaced data from a randomized, double-blind, placebo-controlled trial of 259 infants indeed indicate that hypoallergenic formula supplemented with GOS/FOS may reduce the risk of atopic dermatitis in high-risk infants.¹⁹⁹ In addition, prebiotics have been reported to improve the skin condition of children with atopic dermatitis.²⁰⁰ These data regarding reducing the risk or treatment of atopic disease are preliminary and need to be confirmed by future trials.

CONCLUSIONS

In this chapter, an overview of the interactions between dietary factors, the intestinal immune system, and the indigenous gut microbiota in health and disease has been provided. Nutrients affect mucosal and systemic immune functions and the gut microbiota. General malnutrition or lack of certain nutrients may result in immunodeficiency predisposing to infectious disease. Indigenous intestinal microbes process dietary antigens and contribute to gut immune homeostasis by excluding pathogens and providing maturational and modulatory stimuli. The intestinal immune system constantly monitors intestinal antigens. Protective immune defenses are launched against potential pathogens, while a number of active processes ensure tolerance toward dietary antigens and the microbiota. Abrogation of this tolerance may result in food hypersensitivity reactions or IBD. Recent advances in understanding the mechanistic basis of these interactions provide means to design specific interventions to restore intestinal homeostasis by supplementation of immunomodulatory nutrients or pre- or probiotics. It is to be hoped that these novel therapeutic and preventive modalities will be refined by basic research on more detailed mechanisms on the molecular level and coordinated rigorously conducted clinical trials in the coming years.

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