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Immunology in the gastrointestinal tract

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The gastrointestinal tract (GIT) comprises the largest immune tissue/organ in the human body. The GIT also contains a dense and diverse community of microbial species (mostly bacteria, but also viruses, and fungi). The relationship between this microbial ecosystem and the human host has co-evolved to maintain mutually beneficial and synergistic functions. Therefore, the GIT is the staging ground for coordinated regulation of host recognition systems, namely innate and adaptive immune responses that are required in order to maintain homeostasis. In essence, the goal of GIT immunity is to recognize and maintain those microbes that are required to support host physiology and recognize and expel microbes that can cause illness or disease.

The gastrointestinal associated lymphoid tissue

The gastrointestinal associated lymphoid tissue (GALT) is itself a constellation of both loosely organized and defined immune tissues (Figure 1). The sites within the GALT provide niches that support the interactions between innate and adaptive immune cells. The GALT contains the largest reservoir of immune cells in the body. These cells are required for maintaining homeostasis within the intestinal tract tissues and responding to external threats. In this way the GALT serves its function as: a barrier against pathogen entry and spread within the host, an inductive site for innate and adaptive immune responses by bringing antigen presenting cells and other innate immune cells in contact with antigen specific lymphocytes, and as a site for lymphocyte proliferation and survival.

Gastrointestinal tract epithelial cells

The epithelial cells (EC) of GIT provide both a physical and immune barrier in the GIT. Indeed, they can be considered to be at the “front line” of immune recognition in the GIT. Mucin produced from specialized cells of GIT forms the mucus layer that serves to sequester GIT bacteria and protect from invasive and potential pathogenic luminal bacteria.

Paneth cells are found interspersed among EC in the crypts of small intestinal villi and nestled next to the intestinal stem cells. The paneth cells produce anti-bacterial factors (alpha defensins, lysozymes) that when released protect the crypt stem cells, ECs, and are also absorbed into the intestinal mucin layer to add to its anti-bacterial defenses. Thus, paneth cells also contribute to regulate host GIT colonization by commensal bacterial species.

The EC of the GIT express extra and intracellular pattern recognition receptors (PRR) for the pathogen associated molecular patterns (PAMPs) expressed by bacteria and viral species. PRR include TLR

(extracellular), NOD, and RIG-I molecules (both intracellular). PAMPs include LPS, peptidoglycan, lipoteichoic acid, flagellin, and ssRNA. Binding to PRRs induces intracellular signaling cascades that activate nuclear gene expression and subsequent release of anti-inflammatory factors (cytokines, chemokines, and defensins), and activate other host autonomous pathways (i.e. autophagosomes). These factors in turn serve to recruit immune cells from the gut-associated lymphoid tissues to help fortify the EC immune response and limit microbial expansion or invasion. Responding immune cells from the GALT include innate effectors (neutrophils, macrophages, dendritic cells, eosinophils, mast cells) and adaptive effectors (T cells and B cells). Coordination of the adaptive immune response depends upon crosstalk between ECs and dendritic cells (DC) and results in the expansion of a variety of T and B cell effectors including IgA secreting B cells, activated CD8 $\alpha\alpha$ T cells, and T regulatory cells. Modulation of TLR and NOD signaling in EC likely is one mechanism that regulates responsiveness to the commensal GIT flora in order to limit unnecessary inflammatory responses to the harmless and beneficial bacteria that comprises the bulk of the gut flora ¹.

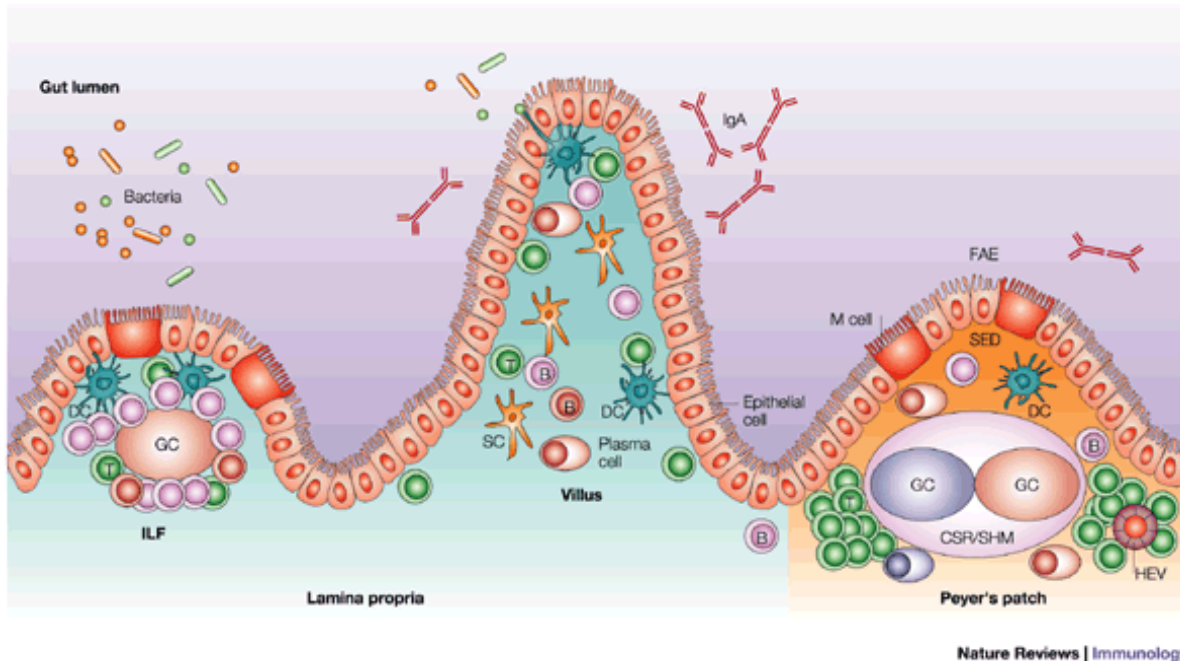


Figure 1. Gut associated lymphoid tissue of the gastrointestinal tract. Reprinted from Nature Reviews Immunology Volume 3(1), Fagarasan, S. and Honjo, T. Intestinal IgA synthesis: regulation of front-line body defenses, pages 63-72, Copyright (2003) with permission from Nature Publishing Group.

The GALT tissues can be generally divided into regions of immune induction and regions of immune effector function². As described in further detail below, the sites of immune induction include the: peyers patches and mesenteric lymph nodes. The site of immune effector function is largely comprised by the lamina propria.

GALT sites involved in induction of immunity

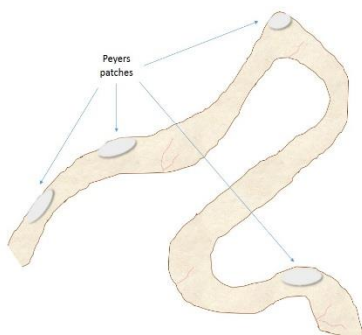


Figure 2. Section of small intestine with arrows pointing to peyers patches.

The Peyer's Patches (PP) are macroscopic lymphoid aggregates that can be seen on the serosal side of the small intestine (Figure 2). In contrast to mice, where PP are found along the entire length of the small intestine, in humans PP are primarily found in the ileum. In humans PP develop prenatally and are apparent by 14 weeks gestation. PP numbers increase after birth. Up to 250 PP can be identified in teenagers; the number decreases to ~100 in senior adults. PP development during embryogenesis requires fetal bone marrow derived lymphotoxin alpha (LT α) expressing cells and the cytokine IL7. Deficiencies in LT α , IL7, or IL7 receptor results in impaired PP development³.

The PP structure includes: follicle associated epithelial cells (FAE), a sub-epithelial dome region (SED), B cell follicles, and T cell zones located between the B cell follicles. In addition, M cells intercalate amongst

the FAE. The PP are not connected to afferent lymphatics so that immune cells traffic from the PP through high endothelial venules (HEV) and then into blood circulation.

The FAE are specialized cells that sample antigens from the intestinal lumen. Dendritic cells (DC; specialized antigen presenting cells) residing beneath the FAE in the SED region capture antigens transported by FAE and M cells to initiate immune responses in T cells. In addition, these DC also can extend dendrites to sample the lumen and capture antigens. FAE express high levels of the chemokine CCR6 that serves to recruit DC and T cells to the SED. Mice with a selective deficiency of the chemokine CCR6 have poorly developed PP and M cells. Moreover, there is a lack of effective recruitment of DC to the subepithelial dome region, subsequent poor responses to oral derived antigens and limited responses against enteropathic viruses ⁴.

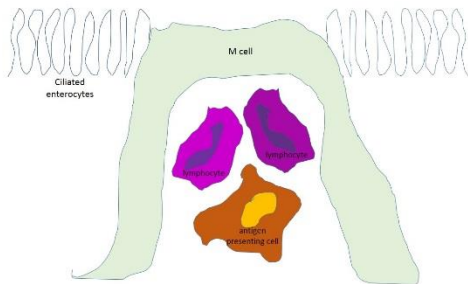


Figure 3. M cell with neighboring immune cells.

The microfold (M) cells in PPs are specialized non-ciliated intestinal epithelial cells that lack a rigid cytoskeleton and intracellular organelles. This allows them to be compressed or deformed so that immune cells and dendrites (the arms of dendritic cells) can migrate into and around them (Figure 3). M cells transport luminal microorganisms and macromolecules to the underlying lymphoid tissues and cells where immune responses are stimulated. *Salmonella* and *Shigella* are examples of two pathogenic microbes that have evolved virulence factors allowing them to attach to M cells in order to facilitate entry into the host. Soluble proteins released by

digestion in the intestinal lumen, are also transported by M cells into the sub-epithelium for uptake by antigen presenting cells or B cells. Thus, M cells support immune responses to oral antigens that result in oral immunization or tolerance.

Isolated Lymphoid Follicles (ILF) are comprised of large aggregates of lymphocytes that resemble PP or lymph nodes. The up to 30,000 ILFs are found scattered along the length of the entire intestine and increase in density from the proximal small intestine to distal colon. ILFs contain B cells (mostly B-2 B cells), CD4⁺ and CD8⁺ T cells, dendritic cells, and IL7R⁺ c-kit⁺ cells. The ILF structures also contain FAE, M cells, a loosely organized germinal center, but no discrete T cell zones. ILF formation is inducible and reversible. Their development is influenced by the intestinal microflora or other inflammatory conditions, like inflammatory bowel disease. ILFs do not develop in germ free mice but emerge after germ free mice are colonized with an intestinal flora. In the absence of ILFs the flora expands 10-fold, thus they also have important function in regulating the density of the intestinal microflora. Induction of IgA in the ILF has a major role in maintaining the homeostasis of the commensal flora. Just like the in the PP, the ILF are sites wherein which adaptive T and B cell responses are induced, including IgA class switching and T cell priming to oral antigens (See review by Eberl and Lochner for further reading ⁵).

The mesenteric lymph nodes (MLN) are located in the mesentery of the small intestine. These are the first lymph nodes to develop during embryogenesis. They require TNF family cytokines for development, unlike other peripheral lymph nodes. Mesenteric lymph nodes serve as a “way station” through which immune cells traffic into and out of the GALT. Thus immune responses detectable in the MLNs may be 1) primed in the intestine, or 2) within the MLNs by antigen bearing DC that migrated from the intestine, or 3) primed by DC that capture free antigen in the MLN that was derived from the intestine. MLN are considered to be critical for induction of oral tolerance to food-derived antigens.

Cryptopatches (CP) located at the base of the intestinal crypts, are loosely organized structures of ~1000 cells. They are identified in the small intestine and colon, but not stomach. Three cell types are found in the CP: lin-ckit⁺ cells, DC, and VCAM-1⁺ stromal cells. CP development is completed after birth in the first few weeks of life, although formation can also occur during adulthood. CP development relies on several factors including: ROR γ t, IL7/IL7R, lymphotoxin (LT), and LT β R. CP support the generation of intraepithelial lymphocytes found in the lamina propria of the intestine. Moreover, CP may also serve as precursors to isolated lymphoid follicles (ILF; for further reading see reference from Newberry and Lorenz ⁶).

GALT sites for effector immunity

Lamina Propria and Intraepithelial Lymphocytes. The diffuse tissues of the lamina propria contain a large number of immunoglobulin A (IgA) secreting plasma cells, B cells, T cells (CD4⁺>>CD8⁺), macrophages, dendritic cells (DCs), and innate lymphoid cells (ILCs). ILCs have recently been identified to have important functions not only in lymphoid development, but also immune regulation (mediated by expression of immune defense factors like Reg3, ROR γ t, ROR α , and AHR), and may also contribute to the pathogenesis of IBD.

The intraepithelial lymphocyte (IEL) population is also large and very diverse in phenotype with function probably based upon distribution of IELs along the length of the intestinal tract (Table 1). In general, IELs all experience a thymic development pathway and mature via thymic dependent (TCR $\gamma\delta$) or independent (TCR $\alpha\beta$) pathways. IELs typically exhibit an activated “antigen experienced” phenotype. The majority of IEL are CD8 $\alpha\alpha$ ⁺TCR $\gamma\delta$ ⁺ T cells. Other T cell phenotypes include: CD8 $\alpha\alpha$ ⁺ TCR $\alpha\beta$ ⁺, CD8 $\alpha\alpha$ ⁺CD4⁺ TCR $\alpha\beta$ ⁺, CD8 $\alpha\beta$ ⁺ TCR $\alpha\beta$ ⁺, Th17⁺, and NK⁺ T-cells.

Table 1. Immune cells in the Lamina propria (LP) and Intraepithelial compartment (IEL)

Cell population	Phenotype	Cytokines Produced	
IELs	TCR $\gamma\delta$ TCR $\alpha\beta$ >80%CD8 $\alpha\alpha$ CD103 ⁺	varied: IFN γ , TNF α , IL4, IL5, TGF β	anti-microbial responses tolerance to oral and intestinal antigens
	NKT		
	Th17	IL17, IL21, IL23	inflammatory responses
	CD4 ⁺ FoxP3 ⁺ Treg	IL10	immune regulation
	CD4 ⁺ FoxP3 ⁻ Treg	IL10	
LPLs	TCR $\alpha\beta$ CD4 ⁺ CD8 $\alpha\beta$ ⁺ CD8 $\alpha\alpha$ ⁺	IFN γ , IL4 IL5	inflammatory responses
	Th17	IL17, IL21, IL23	
	CD4 ⁺ FoxP3 ⁺ Treg	IL10	oral tolerance
	ILC (LTi-like & ILC22)	IL22	immune regulation inflammation
Lamina Propria DC and	CD11b ⁺ CD103 ⁺	TGF β , IL6	sample gut lumen

macrophages	CX3CR1+	retinoic acid	Treg induction IgA class switching
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IgA and secretory IgA (sIgA)

IgA is the most abundant antibody isotype in the serum and in mucosal sites. IgA serves to protect the host by contributing to maintenance of intestinal epithelial barrier function and homeostasis. In humans, two subclasses of IgA are produced: IgA1 and IgA2. IgA1 is found in systemic and mucosal sites, including the lung and intestine. IgA2 is less resistant to degradation by bacterial proteases and is found at greatest abundance in the colon.

Functions of sIgA include:

- Neutralization of microbial toxins and other inflammatory microbial products,
- Binding to invasive pathogens (for example *Shigella* and *Salmonella*),
- Confining commensal bacteria to the mucus layer of the intestinal lumen
- Uptake of luminal antigens and facilitation of excretion of antigens from the LP into the lumen
- Neutralization of antigens in epithelial cell endosomes

The mechanisms involved in the induction of the intestinal IgA response are still not fully elucidated. Colonization of the intestine promotes development of the IgA B cell repertoire via mechanisms that involve dendritic cell activation. M cells can transfer commensal bacteria to dendritic cells in Peyer's patches. Alternatively, dendritic cells directly sample commensal bacteria by extending their dendrites into the bacterial lumen. These bacterial laden dendritic cells can then traffic to MLN to present antigen to plasma B cells with resulting IgA production⁷.

B cells within the germinal centers of PP and MLN are activated via T cell dependent (requiring cognate T cell help) or T cell independent pathways. T cell independent activation of IgA includes TLR engagement on B cells and release of B cell activating factors (BAFF, APRIL) produced by EC or innate immune cells⁸. Activation results in B cell proliferation and differentiation from IgM to IgA producing B cells (known as Ig class switching), and also requires the cytokines IL4, TGF β , and IL6. Further maturation into IgA secreting plasma cells occurs mainly in germinal centers of the PP, but also in MLN, and the LP⁹. T cells (including regulatory T cells) appear to be required for regulating the size of the pool and the survival of IgA plasma cells in the intestine.

Secretory antibodies bind to the polymeric Ig receptor (pIgR) that is expressed on the basolateral surface of the intestinal epithelial cells. Following binding, IgA is endocytosed, pIgR is cleaved, and sIgA is transcytosed to the apical side of the cell and into the lumen. sIgA also binds to Fc α R1, which is expressed on innate cells. Monomeric binding to Fc α R1 results in generation of inhibitory signals whereas cross-linking of Fc α R1 results in activation (antigen presentation, phagocytosis).

Despite the important functional contributions of IgA and sIgA to intestinal homeostasis, its deficiency is not severely detrimental to the host. This is due to compensatory mechanisms provided by intestinal sIgM which also binds pIgR for translocation into the intestinal lumen and other innate pathways. However, the impact of sIgA on limiting innate immune responses, modulating the compartment of intestinal regulatory T cells, and regulating the commensal flora may influence the development of autoimmunity and allergy associated with IgA deficiency.

The intestinal flora

As mammals we can be considered a super-organism. We are host to millions of bacteria, viruses, and fungi that have co-evolved to exist with us and colonize multiple surfaces of the human host (skin, lungs, vagina, GIT). The intestinal tract harbors the greatest proportion of the microbes that colonize the human host, with the colon harboring the greatest density. We derive benefit from these passengers. They also derive benefit from us. Cooperative relationships have established through co-evolution to maintain homeostasis. The GIT flora is comprised of greater than 1000 different bacterial species the majority (>85-90%) of which are non-culturable¹⁰. The density of the flora increases proximal to distal with the greatest density of bacteria found in the colon (up to 10^{12} CFU/ml). The majority of the bacteria (and gene functions) in the intestinal tract have been identified using high throughput gene sequencing and metagenomic analysis. The 3 major groups or phyla of bacteria in the human (and mouse) intestinal tract are the Firmicutes (*Clostridium*, *Eubacteria*), Bacteroidetes (*Bacteroides*), and Proteobacteria (*Enterobacteria*).

The GIT microbiome establishes during the first week of life following birth after contact with the maternal vaginal, intestinal, and oral flora. Distinctions in the flora have been made between infants delivered vaginally vs. cesarean section. The flora undergoes profound shifts during the first few years of life, due to alterations in diet and “environmental experience” in the infant and toddler through fecal-oral routes of exposure and exposure to a variety of infectious pathogens (including viruses and parasites). There is increasing evidence gathered through prospective analyses in the human microbiome project that the intestinal flora stabilizes by 3 years of age into an individually distinct core community of microbiota uniquely determined within each individual.

We have co-evolved with the bacteria in our GI tract and gain significant benefit from them. Well-ascribed functions of the GIT flora include: pathogen displacement, immune system development, nutrient acquisition, vitamin production (e.g. Vitamin K). The germ free mouse has provided a useful in vivo model system from which the benefits of the GIT microbiome can be illustrated. Germ free mice are born and raised without an intestinal flora. In these mice intestinal and systemic immune tissues are deficient in morphology and composition. The immune tissues are smaller and the total numbers of immune cell populations are also lower. When germ free mice are colonized with a complex microflora maturation of immune tissues proceeds¹¹.

The stable differentiation, expansion, and maintenance of specific immune cell populations with have been shown to be dependent upon intestinal microbial colonization. Immune cell populations that are dependent on colonization include: Intestinal CD4⁺FoxP3⁺ Treg cells, Th17 cells, and $\gamma\delta$ ⁺T cells. Strikingly, deficiencies in these cell populations, as noted in germ free mice or as induced by treatment with antibiotics to alter the intestinal flora, are also associated with immune dysregulation and the development of allergic or autoimmune diseases (i.e. type 1 diabetes, rheumatoid arthritis, inflammatory bowel disease) in a number of mouse models. These experimental observations indicate that the intestinal microbiota has broadly-distributed effects on not only mucosal but also systemic adaptive immune function and regulation.

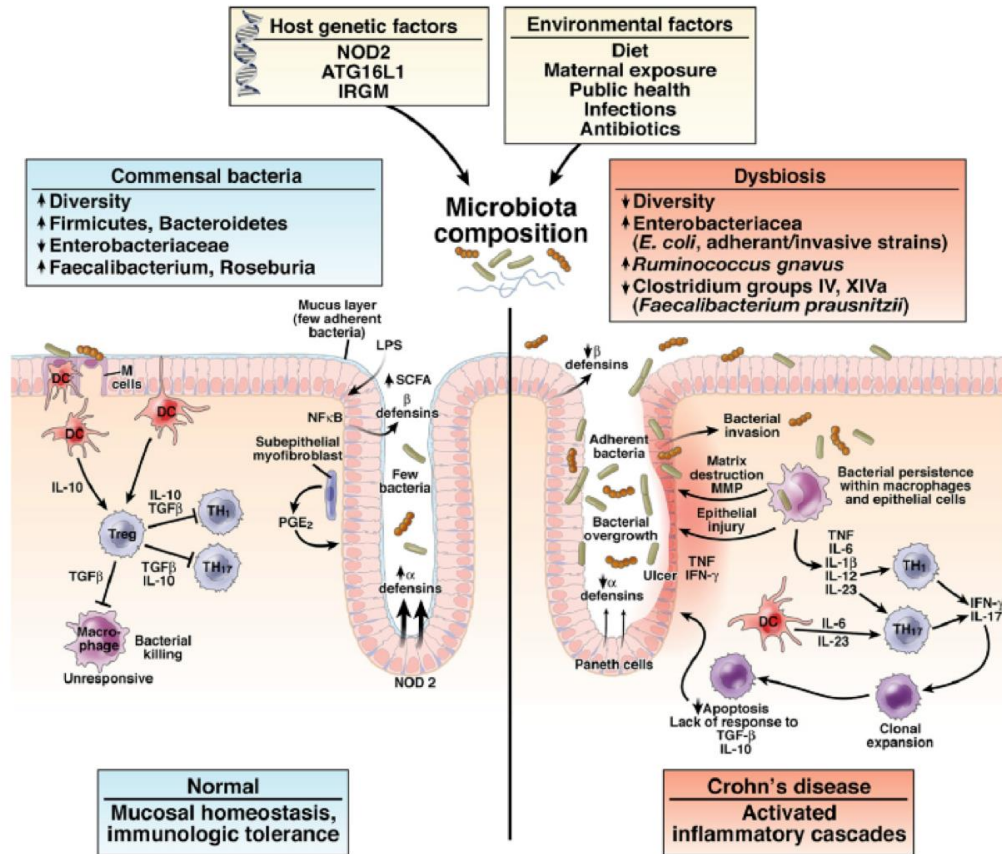


Figure 4. Alterations in the GIT flora influence mucosal homeostasis and immunity. Reprinted from Gastroenterology Volume 139, B. Sartor, Genetics and Environmental Interactions Shape the Intestinal Microbiome to Promote Inflammatory Bowel Disease Versus Mucosal Homeostasis, pages 1816-1819, Copyright (2010) with permission from Elsevier.

When the GIT flora is altered, alterations in host immune cell populations and their function may contribute to dysregulation and the potential for inflammation and disease progression. Dysbiosis generally describes a state of the intestinal flora when it has been altered from a healthy equilibrium or baseline. With the right genetic background or additional environmental insults, diseases like inflammatory bowel disease, diabetes, NAFLD, severe allergies, may thus be promoted (Figure 4). For instance, patients with IBD have less diversity of their flora and increased colonization of the ileum with enteroinvasive *E. coli*. This dysbiosis may develop due to deficiencies in factors that normally regulate mucosal immune responses and subsequently also affect immune mediated regulation of the composition of the GIT flora. These factors include NOD2 expression, paneth cell defensins, and the IL-23-Th17 pathway. Interestingly, the expression of these proteins may be compromised by gene mutations that have been associated with the development of IBD. The consequence of these alterations both in the flora and in mucosal immune function contributes to disease pathogenesis.

Immune tolerance to the commensal GIT flora is required to maintain homeostasis and prevent mucosal inflammation. Multiple mechanisms have been proposed for how this tolerance is generated and maintained^{8, 12}:

- IgA containment & paneth cell defensins
- Treg and tolerogenic DC inhibition of adaptive immune cells (*for instance, control of Th17 cells*)
- Different levels of and cellular distribution of TLRs on ECs
- TLR inhibitor protein (Tollip) expression within ECs
- NOD2 modulation of TLR signaling (*thus, mutations of NOD2 may contribute to IBD*)
- Commensal bacteria mediated inhibition of NF- κ B signaling (*likely through soluble mediators released by bacteria*)
- Molecular mimicry by resident bacteria

Oral tolerance

Ingested food proteins may reach the intestinal tract fully intact for further degradation or already digested into antigenic particles. The lack of immune response to these proteins and antigens is the hallmark of oral tolerance. While some of the mechanisms contributing to oral tolerance remain uncharacterized, the requirements for certain intestinal immune tissues and cell populations have been described.

In general, tolerance to food proteins results in a lack of subsequent intestinal and systemic immune responses. This is unlike tolerance induction to bacteria in the colon where local responses are suppressed but systemic immune responses against bacteria are not affected. Uptake of soluble food antigens occurs via several routes¹³:

- transcytosis through M cells,
- paracellular diffusion or transcytosis across intestinal villi ECs,
- uptake of exosomes (MHC Class II loading compartments fused with endosomes) by lamina propria DC,
- luminal capture by CXCR31^{high} DC/macrophages.

In all cases, antigens are eventually captured or transferred to migratory CD103⁺ DC in the lamina propria. These DC transport antigen to the mesenteric lymph nodes, the main site for oral tolerance

induction. These DC have the specialized ability to metabolize dietary Vitamin A (retinoic acid; RA) that induces the selective expression of gut homing receptors $\alpha 4\beta 7$ integrin and CCR9 on activated antigen specific T cells. In addition RA along with TGF β induces the conversion of peripheral naïve CD4 T cells into FoxP3⁺ T regulatory cells (Tregs). The migration of FoxP3⁺ Tregs back to the lamina propria and IL-10 cytokine mediated expansion is probably required for maintaining long-lasting oral tolerance. It is also interesting to note, that stromal cells of the MLN express RA and TGF β , and thus also have an important shared contribution to supporting the mechanisms and cell populations required for oral tolerance induction.

Exactly how and by what mechanisms tolerance to food antigens is systemically maintained is still murky. There is evidence for contribution from liver derived antigen presenting cells (Kupffer cells and conventional DC), peripheral lymph node antigen presenting cells (specifically plasmacytoid DC).

Neonatal intestinal immaturity and mucosal immune function

The infant intestine is underdeveloped in multiple aspects that govern host mucosal immunity. First, barrier function is weak, in that there is increased intestinal permeability noted. In humans, decrease in intestinal permeability and permissiveness to macromolecule transport occurs within the first few days of life. This “gut closure” as it has been termed, is promoted by maternally derived EGF and TGF found in breast milk and colostrum¹⁴. In addition, breast milk contains antimicrobial factors such as lactoferrin and lysozymes both of which inhibit bacterial and viral pathogens. The digestion of breast milk by lingual and gastric lipases also releases free fatty acids and monoglycerides that are toxic to some viruses and parasites.

The generation and expansion of B and T cell populations in the intestinal peyers patches and lamina propria is deficient in neonates. However, maternal IgA secreted into breast milk and consumed by the infant can make up for the deficiency. Secretory IgA is bound to polyimmunoglobulin receptor (pIGR) to cross the mammary gland cell where it is cleaved and released in milk. IgA is digested by the infant and passes through to the intestine where it traps food antigens and inhibits bacterial and viral invasion or colonization of the intestinal tract. In addition, IgG is transferred in humans in utero across the placenta and after birth in the breast milk via immune complex transfer mediated by binding to the neonatal Fc receptor (FcRn) molecules expressed on mammary and proximal small intestinal ECs. Transfer of complexed antigens into the lamina propria supports immune responses including generation of Tregs, thus supporting oral tolerance induction in infants.

Diet and microbial colonization support immune tissue development and function following birth. As well demonstrated in germ free mice, a lack of intestinal microflora results in poorly developed intestinal and systemic immune tissues and deficient immune cell populations¹¹. The effect of diet is also noted from observations in infants receiving parenteral or amino acid based nutrition. These infants have smaller thymuses, deficient mucosal lymphoid tissue development, and reduced numbers of T and B cells in the IEL and lamina propria. Breast milk oligosaccharides and glycans have a prebiotic effect, specifically promoting the expansion of *Lactobacillus* and *Bifidobacteria* species in the infant intestinal tract. Metabolism of milk sugars by these bacteria results in an acidic luminal pH, which inhibits colonization by harmful bacteria¹⁵. The presence of these bacteria may also have other effects on mucosal immunity, including supporting EC barrier function and allergy prevention (although the exact mechanisms in the latter are not delineated). There is some evidence that the threshold for stimulation of inflammatory responses in infants may be low, thus the risk for sepsis and intestinal inflammation is higher. Anti-inflammatory cytokines include IL10 and TGF β . Breast milk contains high concentrations of TGF β , which augments the low levels of this down-regulatory cytokine in the intestinal tissues of

neonates. Thus, this may help to regulate inflammatory responses that are stimulated in the intestinal tract of neonates by pathogens or food antigens. Other immune factors isolated in milk that support immune homeostasis in the immature neonatal gut include: soluble TNF α receptors, IL-1RA, and soluble CD14.

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1. A 10 year old girl presents with abdominal pain and poor growth. Laboratory testing reveals an elevated anti-tTG IgA (25 U/ml) and normal anti-endomysial IgA. Her serum IgA is noted to be 6mg/dl. Celiac disease is confirmed by endoscopy. In some patients IgA deficiency may also be associated with:

- a) increased risk of developing juvenile rheumatoid arthritis
- b) frequent sinus infections
- c) hives after she eats strawberries
- d) none of the above
- e) all of the above

2. A 17 year old patient presents with bloody stools, a history of intermittent fevers and abdominal cramps. A colonoscopy confirms ulcerative colitis and his disease responds to initial treatment with steroids and remains in remission with 6MP. He decides to take a preparation of 8-probiotics because he read somewhere that "bad flora" could contribute to his disease. The factors that regulate the density and composition of the intestinal flora include:

- a) Paneth cells
- b) T regulatory cells
- c) Th17 cells
- d) ILCs
- e) None of the above

Answers:

1. E

Patients with selective IgA deficiency can be completely asymptomatic (80-95%) or can have an increased risk for autoimmune disorders (including celiac disease), sino-pulmonary or intestinal infections, and food allergies.

2. A

Paneth cells, probably via secretion of anti-bacterial products, have been shown to regulate the density and composition of the colonizing intestinal bacterial flora. The other listed factors have not yet been shown to have a direct effect on regulating the intestinal flora. Colonic T regulatory cells and Th17 cells depend on specific bacterial species in the intestine for their development.