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The Roles of Epithelial Cells in Gut Immunity

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Abstract

The epithelial layer of the gastrointestinal tract are the columnar cells exposed to the lumen, the site where food matter and food-borne pathogens come into contact with our body. As they are the front line against food-borne illness, they are responsible for sensing the luminal environment and modulating secretions they produce in response. Gut epithelium operates a melody of ways in which it provides a barrier, targets pathogens, promotes our mutualistic microbiota and induces long lasting immunity against infection, all the while allowing us to absorb nutrition. This review examines the role of gastrointestinal epithelial cells in maintaining gut health and tolerance.

Keywords: Gastric, Intestinal, Epithelial cells, Immunity.

INTRODUCTION

Intestinal epithelial cells have an important role in the maintenance of a healthy gut barrier. Intestinal epithelial cells are involved in immune-regulation essential for tolerance and in anti-pathogen immunity. Intestinal epithelial cells transport secretory immunoglobulins across the epithelial barrier. Plasma cells in the lamina propria produce dimeric IgA complexes which bind to the receptor of polymeric immunoglobulin (pIgR) on the basolateral membrane of intestinal epithelial cells, and are transposed into the intestinal lumen [1]. The relationship between IgA-secreting B cells and intestinal epithelial cells delivers the component of an adaptive immune to the epithelial barrier which regulates bacterial commensal populations to maintain homeostasis to intestinal epithelial cells and immune cells [2, 3].

The ABO blood group system is one of the genetically determined host factors modulating the composition of the human intestinal microbiota but pathogenic bacteria and viruses have been shown to use ABO blood group antigens as adhesion receptors [4]. There is an important relationship between the blood group secretor status, cruciallydetermined by the activity of fucosyltransferase-2, *FUT2,* gene, and the composition of the intestinal flora. A study of secretors and non-secretors revealed a significant association with altered compositions of bifidobacteria, with likely relevance for susceptibility or severity of intestinal inflammation [5]. The ABO blood group antigens are present in saliva and are also expressed in the intestinal mucosal layer. They act as adhesion molecules or energy sources for intestinal microbes, yielding host-specific microbiota composition [6, 7]. In fact, the development of ABO antibodies in newborns [8] follows the colonisation with gut microbiota [9].

The commensal bacteria and intestinal epithelial cells interact using pattern recognition receptors (PRRs), e.g. Toll-like receptors (TLRs). TLRs are able to initiate the inflammation in response to microbial insult. Intestinal homeostasis is established by discriminating between symbiotic commensal bacterial and harmful pathogens through innate immune receptors [10]. TLR signalling recognises the composition of the local microbiota this lead to the underlying effector and regulatory immune cells, and reduces inflammation while providing immune protection [10, 11, 12].Therefore, commensal bacteria cross-talk with intestinal epithelial cells is essential for regulating the activation levels of NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and maintaining intestinal homeostasis. NF-κB is a protein complex pathway that are involved in e.g. the immune and inflammatory responses, cellular growth and apoptosis, also active in a number of disease e.g. cancer.

Vitamin K is synthesized by small intestinal microflora and is an essential cofactor in the production of prothrombin and other blood clotting factors and immunity. The Vitamin K are groups that consists of Vitamin K1 and Vitamin K2. While Vitamin K1 is synthesized by plants, Vitamin K2 can be formed

to Vitamin K1 through most of the microflora of the gut and microbiota species, e.g. *Enterobacter sp*, *Eubacterium lentum*, *Veillonella sp.* and *Bacteroides sp.* [13]. The lipophilic vitamin is absorbed in the small intestine using bile salts [14].

Sensing of microbiome

The intestinal mucosa mounts innate responses to protect the host from infection with enteric bacteria. Important mechanisms are: the sensing of microbiota using membrane bound and intracellular PRRs (including TLRs and NLRs), amplification of pro-inflammatory responses, the secretion of antimicrobial proteins and the recruitment of neutrophils. In the gut the interaction between commensal bacteria and host cells is tightly regulated to distinguish commensal microorganisms from pathogens. TLR-based recognition of commensal bacteria represents supports the successful symbiotic relationship between the host and its microbiota. In the intestinal epithelium cells the TLR signalling has a beneficial role in maintaining intestinal homeostasis. Several Toll-like receptors are expressed in human small intestinal epithelium cells including TLR1, TLR2, TLR4, TLR5 and TLR9 [15]. It has been shown that TLR signalling is involved in epithelial cell proliferation, IgA production, maintenance of tight junctions and antimicrobial peptide expression, essential for maintaining a healthy epithelial barrier. However, in the normal state of the intestine, TLRs expression by epithelial cells are usually low, however, during inflammation it increases, as happens e.g. in colonic crypts of patients with ulcerative colitis and Crohn's disease and on the villi of patients with ileal Crohn's disease [16].

Commensal bacteria induce the expression of the antimicrobial lectin regenerating islet-derived protein 3γ (REG3γ) in Paneth cells in response to TLR stimulation. REG3γ specially targets Gram-positive bacteria by binding to their surface peptidoglycan [17, 18].

Stimulation of TLRs of intestinal epithelial cells also impacts on the local adaptive immune reactions: TLR-mediated expression of proliferation-inducing ligand (APRIL) promotes class switch recombination (CSR) of IgM and IgA1 to protease resistant IgA2. This is important because enteric pathogens are able to proteolytically degrade this mucosally secreted immunoglobulin. Normally, IgA2 prevents bacterial invasion by binding bacteria at the apical surface of intestinal epithelial cells. In addition, the activation of TLR2 promotes the movement of cells by stimulating the production of trefoil factor 3 (TFF3) essential to repair gaps in the epithelial monolayer, which is termed restitution. The cross-talk between bacteria and intestinal epithelium cells through myeloid differentiation primary-response protein 88 (MyD88)-dependent signalling is essential for the maintenance of gut homeostasis. Mice deficient of TLR and MyD88 develop more severe disease than their wild-type littermates. Whether or not Paneth cells use TLRs in a direct response to the presence of bacterial products is not yet known. In ex vivo studies of intestinal epithelial cells isolated from mice deficient of TLR2 and TLR4 or signalling of MyD88, an important role for TLR signalling is found for the regenerative proliferation of intestinal epithelial cells.

Sensing of pathogens

A study by Boullier *et al*, 2009 examined the effect of inoculation of *Shigella flexneri* into mice both with and without a dose of secretory Immunoglobulin A (sIgA) large enough to cause complete agglutination. When *S. flexneri* was inoculated alongside sIgA this caused higher expression of the anti-inflammatory cytokine IL-10 from Peyers patches, as well as reduced expression of the pro-inflammatory cytokines Cox-2, TNF-α, IL6 and IFN-γ. Initially this may seem to be the opposite of sIgAs normal role, however, *S. flexneri* causes severe localised inflammation and rupture of the colonic mucosa, which it then takes advantage of to further its own invasion into tissues. This reduction in inflammation through the control of cytokine expression means that the integrity of the epithelial barrier is maintained and the virulence of pathogens which capitalises on inflammation is lessened. This shows that sIgA is capable of effects not present in other immunoglobulin classes, which help to protect the tissue environment [19].

Defensins are able to agglutinate pathogens, localising them together and increasing the ease of their destruction by the immune system or ejection from the body. However, these small peptides can have an effect not only on the number, but also the phylogenetic makeup and localisation of the microbes present in our gut. Transgenic studies in germ free mice using the human defensins HD5 and HD6 highlighted these interesting effects [20]. Firstly they showed that the gastric microbiota of mice transgenically imbued with HD5 was significantly different from that of germ free mice imbued with no defensins. There was a significant decrease in Firmicute and Actinobacteria species while the number of Bacteriodes species increased by a large margin. The Firmicute phylum contains species such as *Clostridium botulinum*, *Clostridium tetanii* and *Staphylococcus aureus*. The Actinobacteria contains genera such as Streptomyces. The Bacteriodes phylum on the other hand contains species such as *Bacteriodes fragilis*. Bacteriodes species are mainly mutualistic to humans and so their increased proportion in lieu of pathogenic phylum would create a safer environment [20].

It has already been mentioned how the microbiota directly competes with pathogens for space and nutrition, however the microbiota is also capable of interacting with the epithelia and affecting cytokine secretion. A study on germ free mice by Atarashi *et al*, 2015 looked at the effect of introducing *Citrobacter rodentium*, a human microbiota species, into mice. What they discovered is that when wild**-**type *Citrobacter rodentium* was introduced, T cells present in the Peyers patch begin to express high levels of both RORγt receptor as well as IL17. IL17 is a chemoattractant for both neutrophils and monocytes while the RORγt receptor is utilised in activation of T cells, specifically TH17 cells [21]. The combined effects of this increased expression is a larger number of immune cells with a higher level of activity in the area, which will enable pathogens to be destroyed more easily. When the study utilised a Citrobacter rodentium mutant strain which has had an essential adhesin removed, we can see that the increased expression has been almost entirely stopped. This indicates that attachment of the *Citrobacter rodentium*, rather than recognition of pathogenic antigens by PRRs or antibodies, caused the activation of the immune system. A range of twenty human gastric microbiota species, including members from the Ruminococcus, Clostridium, Firmicutes and Bacteriodes phyla, were isolated from clinical faecal samples and subjected to this experimentation and all of them produced a similar effect. This shows that while our body provides a warm environment and a site of nutrition for many bacterial species, the relationship is not one-sided in any regard and in fact may benefit us more than them [21].

Innate Immunity along the gastrointestinal tract

Chief cells in our stomach release a large number of degradative enzymes into the gastric lumen to break them down. Some of these enzymes are gastric proteinases such as pepsin which degrade peptide chains into smaller stretches of amino acids that can be absorbed by our intestinal cells. These enzymes, which are secreted as inactive zymogens, exhibit optimal activity at the acidic pH our gastric tract is maintained. The activated zymogen carries out protein cleavage which aids our digestion. Gastric proteinases also enact innate immunity as they digest the extracellular face of any bacterial transmembrane proteins. As their surface proteins are digested, this prevents them from carrying out their function which could have profound effects on the virulence of the bacteria by reducing its motility, preventing epithelial adhesion or degrading receptors important in stress sensing [22].

Parietal cells in the epithelium split carbonic acid into protons and bicarbonate ions using the enzyme carbonic anhydrase. The protons are then secreted into the gut lumen using a proton pump which uptakes potassium ions in return, these potassium ions then re-enter the gut lumen through a potassium channel. The pumping of protons into the gut lumen decreases the pH which activates digestive zymogens and breaks down electrostatic interactions which causes protein unfolding, this aids digestion and also reduces pathogenicity of microbes. Finally, the bicarbonate ions produced are secreted into the lumen and act as a final barrier between the epithelia and the acid. If the acid penetrates the mucus, then the bicarbonate neutralises the acid and epithelial damage is avoided [23]. Proton pump inhibitors (PPIs) are a class of drugs used to treat gastric acid-related disorders, for example gastroesophageal reflux and peptic ulcer disease, and reduce the production of acid in the wall of the stomach. Proton pump inhibitors act by blocking gastric acid secretion mainly via irreversible inhibitors of the H^+/K^+ ATPase pump [24], and thereby need to be viewed with caution as far as the innate barrier of the stomach is concerned. Figure 1 shows all of the Innate Immune barriers present in our stomach [25].

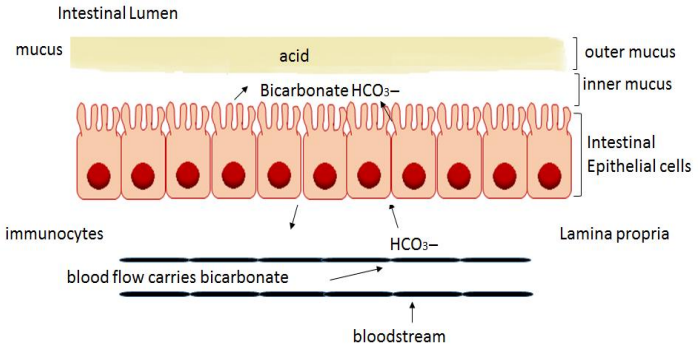

Figure 1: Diagram showing the Innate Immune barriers which maintain gastric immunity

Epithelial cells ensure microbes do not enter our tissues by forming a continuous, almost impregnable physical barrier that surrounds the gut lumen. This barrier is maintained by tight junctions between the cells formed by the proteins claudin, occludin and the epithelial cell adhesion molecule (Ep-CAM), all of which are found in the epithelial cell membrane [25]. If these tight junctions were not maintained then the epithelial barrier could fail and pathogens present on ingested food could easily invade our tissues. The epithelial barrier also maintains the polarity of the epithelial apical and basolateral membranes by preventing surface markers such as receptors and ion channels from diffusing to the opposite membrane. This ensures the functions of these molecules occurs unimpaired which allows correct signalling to occur and prevents the uncoupling of ion flow across the membranes [26].

The epithelial layer of the gut is organised into 3D structures called villi, in between these villi are crypts where epithelial cells are generated and as they age and differentiate they progress to the villi tip. Figure 2 shows this structure and the villi greatly increase the epithelial surface area, increasing the contact between our body and ingested material. Intestinal epithelia also has a very short turnover time of two to four days. After this they undergo apoptosis and shed into the gut lumen, continual shedding of epithelia prevents long-lasting infections from taking hold and causing damage, as well as reducing the risk of microbes remaining attached to the epithelia for long periods [27].

The balance between the average of apoptosis and shedding of senescent epithelial cells at the villus tip, and the generation of new cells in the crypt is the key to maintain tissue homeostasis. Excessive shedding or cell loss of intestinal epithelial cells from the epithelial monolayer may increase intestinal permeability. In many intestinal diseases, shedding from the villus exceeds the regenerative capacity of the crypts, due to epithelial injury to one or both components. It is interesting to note that Host-microbial interactions, be they between host and commensals or host and pathogens, influence epithelial permeability, intestinal epithelial apoptosis related to cell shedding [28]. Irradiation induced diarrhoea is due to the effect on the high proliferative capacity of epithelial cells in crypts and their subsequent loss [29].

Paneth cells are intestinal epithelial cells highly specialised for the synthesis and secretion of antimicrobial peptides (AMPs). They store these peptides in cytoplasmic granules near the apical membrane until the secretion stimuli is received, which may be the activation of a Toll-like receptor, at which point the granules undergo exocytosis into the intestinal lumen [30]. Some of the AMPs secreted by Paneth cells include α-defensins, lysozyme and secretory phospholipase A2. The human α-defensin HD6 attaches to bacterial surface molecules such as flagella or fimbriae and then further HD6 molecules bind to the first HD6 molecule that attached. More and more HD6 molecules bind on and this builds a network interlinking fibres, forming a peptide web that surrounds bacterial cells and traps them [20]. Paneth cells are also unique as intestinal epithelial cells in the respect that as they specialise, they migrate towards the crypts instead of the villi tips as the other intestinal epithelia cell types do. This causes the concentration of the defensins and other immune molecules to be highest in the crypts which prevents bacterial species from surviving in these sites. This will instead localise bacteria closer to the villi tips, as these are extended into the gut lumen. This prevents infections from occurring in the crypts where they are surrounded by tissue and could easily spread, however, if an infection of a villi tip occurs it has to progress through the entirety of the villi to reach other tissues [30].

Phospholipase A2 cleaves phosphatidylglycerol, a major component of bacterial membranes. This destabilises the bacterial cell membrane and makes it more likely to collapse and lyse the cell [31]. Lysozyme is a degradative enzyme capable of digesting peptidoglycan and chitin which are components of bacterial cell walls and cyst cell walls respectively. Lysozyme degrades these cell walls which increases the likelihood the cell wall collapse in on itself as it cannot maintain its shape. Lysis of a cell wall results in death of the cell as it releases all intracellular molecules with no way to localise them together, uncoupling cellular metabolism [32].

Goblet cells in the intestinal epithelia secrete several compounds which work together to form a dense gel-like component called stomach mucus. This is made up of the mucins, MUC2 and MUC5AC which are highly glycosylated and form a sticky polymeric gel like substance around the gut surface. Epithelial cells of the gut also produce the transmembrane mucins MUC3, MUC12 and MUC17 on their apical surface which form the glycocalyx, which protrudes around 1µm into the gut lumen and is coated in glycan molecules. As we do not produce any glycosidases that can degrade these mucins, this means the digestive enzymes and acid secreted into the lumen are unable to damage the epithelia layer. The sticky nature of the mucus traps bacteria within it and as the mucus is expelled from the gastric tract by peristalsis, so are the trapped microbes. The mucus also captures AMPs, creating an AMP gradient that is highest near the epithelial layer, this will reduce the number of bacteria able to make contact with the epithelia.

There are different sugar degrading enzymes, these enzymes are released by cells in the small intestine, and works on a different type of sugar e.g. sucrase which divide the sucrose to glucose and fructose, also lactase which divide the lactose to glucose and galactose, moreover, the maltase divide the maltose to glucose, and the dextrinase divide the chains of glucose to individual glucose units. Short-chain fatty acids (SCFAs) are the products of the bacterial fermentation of undigested carbohydrates in the intestine [33, 34]. This production in the intestinal lumen is important for normal intestinal

biology. SCFAs help numerous essential roles in the intestine to modulate immune responses such as regulate ion absorption and gut motility [35]. SCFAs activate several G protein–coupled cell surface receptors and GPR109a, a receptor expressed by gut epithelial cells, and other cells such as adipocytes, macrophages, and dendritic cells. SCFA (such as butyrate) promote apoptosis of T-cell and reduce accumulation of T cells by up-regulating Fas in inflamed colonic mucosa [36]. Fas-FasL interaction is an important effector mechanism to uphold intestinal immune tolerance. The human commensal bacterium *Bacteroides fragilis* produces a carbohydrate polysaccharide A which is a dominant factor in the improvement of T cell–driven colitis in an IL-10–dependent manner [37]. Enteropathogenic bacteria interfere with tight junctions, mucin decoration and ion channel activities which cause diarrhoea. *Vibrio cholera* and enterotoxgenic *Escherichia coli* produce virulence factors including toxins which act on the small intestine to cause outpouring of fluids into the lumen, and acute inflammation, dysentery.

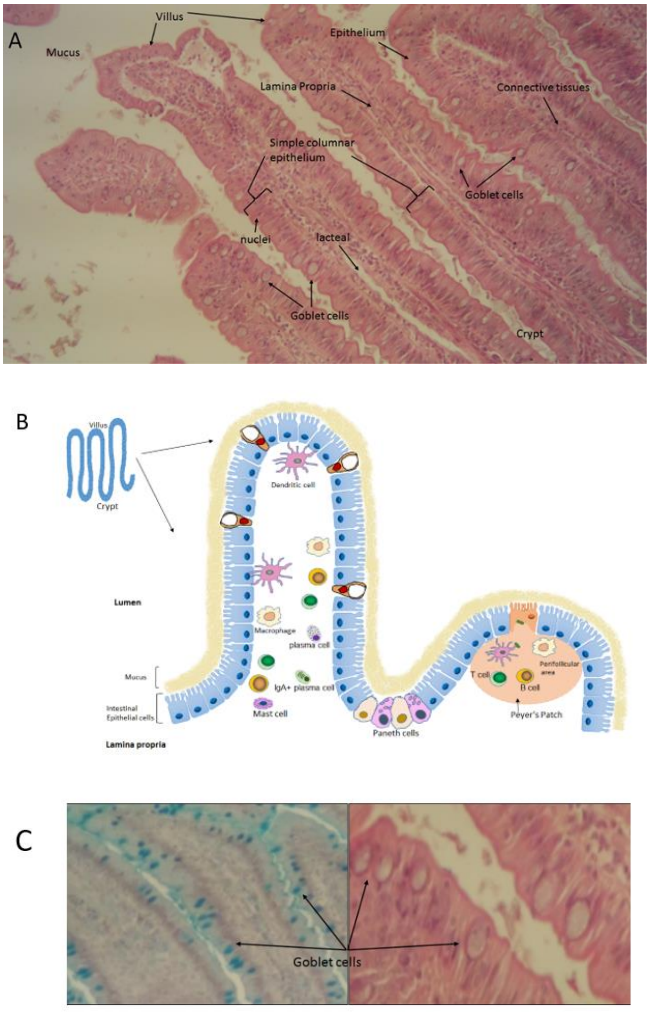

Figure 2: Our intestinal Epithelia is organised into folds which form 3D structures such as crypts and villi. A. histological image of jejunum B. Cartoon of ileum. The insert highlights goblet cells, Alcian blue positive, and in hematoxylin/eosin stain.

Adaptive Immunity in the Gut

Adaptive immune cells include T cells, B cells and plasma cells whose activation leads to the production of sIgA protective. In addition, the intestinal microbiota provide a safeguard to infection, during competition for nutrients and space during modulation of mucosal immunity [38].

The immune system of the intestinal mucosa is composed of an assortment of lymphocytes, e.g. IgA- generating plasma cells, IELs, γδT cells and IFNγ generating CD4+ T (Th1) cells. Moreover, current studies show the existence of innate lymphoid cells and Th17 cells, also Treg cells, NKp46+ cells and LTi cells in the gut. Till now it is not fully understood the mechanism by which the intestinal mucosa harbours diverse sets of lymphocyte populations, but is likely to be related to the particular cytokine environment (IL1-, TGFb rich). In addition, the intestinal mucosa encloses several IgA-secreting plasma cells, and secreted IgA plays an important part in the host defense versus pathogenic bacteria. Significantly, IgA regulates the composition of bacteria microflora of intestine [39].

Antibodies are one of the most potent effectors of our immune system and this protection has been extended to the gut lumen. After antigens have been sampled by the gastric associated lymphoid tissue (GALT), which will be explained further, this induces a humoral response from GALT associated B cells which undergo class switching and begin to produce sIgA. sIgA secretion begins by binding of IgA to the polymeric Ig receptor. The IgA is then translocated across the basolateral membrane of the epithelial cells and the extracellular region of the polymeric Ig receptor, called the secretory component, is cleaved from the surface and is expelled into the gut lumen. This complex of secretory component and IgA is the sIgA which is released from our epithelial cells. The secretory component has a strong propensity to bind to mucus and this is beneficial as it traps pathogens in the mucus which will passed through the body, preventing pathogens from ever interacting with the epithelia [19].

sIgA shares many features with other Ig classes, such as the ability to bind and block toxins, preventing them from damaging host tissues. It can also bind to the surface molecules of pathogens and prevent them from carrying out their function. If this surface molecule is essential for adhesion of the pathogen to the epithelia then attachment will not occur and the pathogen will be expelled with faeces. Agglutination can also occur which is when sIgA molecules crosslink large numbers of pathogens into a single unit using the two antigen binding sites. Agglutination can aid phagocytosis by gut resident dendritic cells via trapping several bacteria together as one unit in close proximity which aids their phagocytosis and means effector molecules only have to localise at one site rather than several distinct sites, which will increase their concentration and efficacy [40]. The secretory component and heavy chain of sIgA are heavily glycosylated in sugars which share a lot of similarity with the sugars on the luminal face of the intestinal epithelia. In this manner sIgA can act as a decoy for pathogens that attach to these sugars. This is further established as addition of the sIgA secretory component without sIgA is able to bind to the intimin adhesin of enteropathogenic *Escherichia coli* and the phosphocholine molecule of *Clostridium perfringens*. By binding to sIgA these surface molecules can no longer bind to their targets and so they have been neutralised [40].

Decision making at the epithelial barrier

While the immune system is designed to destroy foreign microbes, there is a large subpopulation of bacteria in our bodies, called the microbiota. The microbiota covers the external and internal surfaces of our bodies and in most cases aid our immunity. The presence of microbiota means that for a pathogen to establish itself, it has to compete with the microbiota for both space and nutrients, reducing their growth rate and preventing severe infections taking hold. The microbiota in our gastrointestinal tract also aid our bodies by digesting chemical bonds in our food that we are unable to digest, increasing the bioavailability of nutrients [41]. Studies which looked at germ free members of several species also found that normal intestinal function and development of the GALT is dependent on the presence of a microbiota [42].

Epithelial cells recognise microbes through Toll-like receptors, Nucleotide Oligomerisation Domain (NOD) receptors and other similar

families which detect microbial ligands. There are many different types of these receptors with 11 TLRs present in our body that when activated induce the expression of NF-κB, a potent pro-inflammatory molecule (Figure 3) [43]. However, both commensal and pathogenic species produce the ligands of these receptors, yet only commensal species are maintained. The reasons for the different outcomes are unknown but thought to relate to the actions of the bacteria. It is thought that in the presence of other inflammatory signals, such as molecules released by tissue damage, TLRs induce an immune response while without these signals the TLRs do not mount this response. Instead they signal through other pathways and maintain normal functions. This was shown in TLR deficient mice mutants which experienced much greater damage from administration of a toxin against the colonic epithelia than mice who had no TLRs removed [44].

Epithelial cells can also induce a type 2 immune response that is characterised as a large number of basophils, eosinophils, mast cells, type 2 innate lymphoid cells and alternatively activated macrophages. Helminthic parasites such as tapeworms can be significantly larger than pathogenic bacteria, this means they are unable to be taken up into immune system cells for phagocytosis. Instead they are degraded extracellularly which will require a long time period and high concentrations of chemokines and degradative molecules. To circumvent this and prevent damage to the host the type 2 immune response induces increased production of mucus and increased peristaltic motion which will immobilise the helminths and pass them through the digestive tract naturally [45].

In the absence of inflammation, epithelial cells will not produce the costimulatory molecules CD80 or CD86, but they can still uptake antigens and present them to T cells. The expression of these molecules during inflammation is less clear [46]. However, some studies report that human intestine epithelial cells do not express CD80 and CD86 during inflammatory bowel disease, but other studies show selective expression of CD86 during active disease in biopsy specimens or with IFNγ-treatment in culture [47, 48]. The ability to present different antigens alongside differing signals to T cells allows the immune system to determine which antigens are harmful and require an immune response. Conversely this also allows consumed food matter to be digested without activating the immune system, establishing oral tolerance [49].

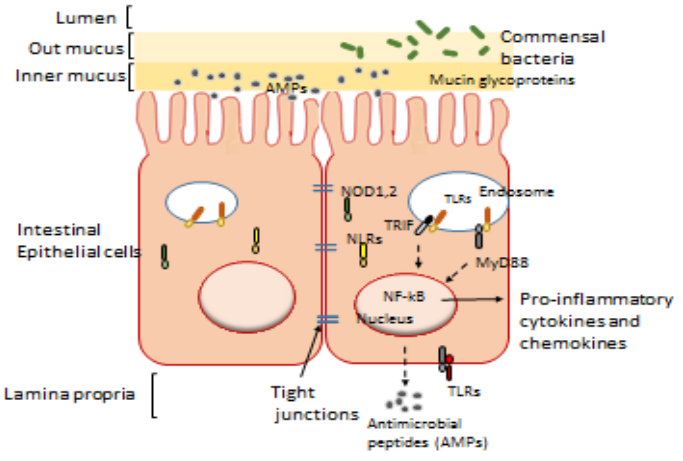

Figure 3: The polarity of gut epithelial cells with expression of receptors luminal/basal

CONCLUSION

The gut is a site of major interaction between our body and external materials. To reflect this the outermost cell layer, the epithelium, is highly specialised in sensing the gut lumen and the microbial species present within. It uses a number of diverse and potent immune mechanisms that ensure this immunity is maintained. These mechanisms have the potential to not only work alongside one another, but also to interact with each other, fine tuning the environment of our gut to promote microbiota species which benefit us, while excluding harmful pathogens. This ensures that our bodies are able to acquire the energy they need in the most efficient manner possible, which is essential for our wellbeing and health.

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