

Th17 Cytokines and the Gut Mucosal Barrier

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Abstract Local immune responses serve to contain infections by pathogens to the gut while preventing pathogen dissemination to systemic sites. Several subsets of T cells in the gut (T-helper 17 cells, $\gamma\delta$ T cells, natural killer (NK), and NK-T cells) contribute to the mucosal response to pathogens by secreting a subset of cytokines including interleukin (IL)-17A, IL-17F, IL-22, and IL-26. These cytokines induce the secretion of chemokines and antimicrobial proteins, thereby orchestrating the mucosal barrier against gastrointestinal pathogens. While the mucosal barrier prevents bacterial dissemination from the gut, it also promotes colonization by pathogens that are resistant to some of the inducible antimicrobial responses. In this review, we describe the contribution of Th17 cytokines to the gut mucosal barrier during bacterial infections.

Keywords IL-17 · IL-22 · gut inflammation · Th17

Intestinal Pathogens and the Gut Mucosal Barrier

In normal individuals, the gut mucosa constitutes a barrier against the systemic spread of both pathogenic microorganisms and the resident intestinal microbiota. Once this barrier

has been breached, dissemination of microbes from the gut can result in a systemic disease known generally as sepsis. With regards to bacteria, the frequency of bacteremia (bacterial sepsis) is higher in patients with altered mucosal immunity (such as children and the elderly) as well as in patients affected by immunodeficiency, implicating the mucosal barrier as an important line of defense against bacterial dissemination [1, 2]. Similarly, increased permeability of the mucosal barrier with concomitant bacterial translocation has been reported in Crohn's disease [2].

A portion of the gut mucosal barrier function is orchestrated by the intestinal microbiota that coexist with the host in a mutually beneficial symbiosis [3]. These functions include inducing secretory IgA production and intraepithelial lymphocyte recruitment as well as providing a physical blockade to pathogen colonization [4]. At least one of these mucosal defenses or others to be detailed below must be circumvented or endogenously fail for pathogens or the resident microbiota to disseminate beyond the gut mucosa.

Some intestinal pathogens including nontyphoidal *Salmonella* (including *Salmonella typhimurium*), *Campylobacter*, and *Shigella* cause inflammatory diarrhea and are characterized by their ability to invade the intestinal mucosa. In stark contrast to these organisms, the closely related pathogens enterohemorrhagic *Escherichia coli* (EHEC) and enteropathogenic *E. coli* (EPEC), among others, cause secretory diarrhea and are noninvasive [5]. With regards to the latter group, these strains of *E. coli* belong to the class of organisms known as attaching–effacing (AE) bacteria because they form characteristic AE lesions as a result of their intimate adhesion with the intestinal mucosa. As both EHEC and EPEC are poorly pathogenic in mice, *Citrobacter rodentium*, a natural mouse pathogen related to *E. coli* which also causes AE lesion formation, has been widely used as an experimental model for AE pathogens and will thus be included in this discussion [6].

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Orchestrating the Gut Mucosal Barrier: an Emerging Role for T Cells

The host innate immune system is activated by pathogen-associated molecular patterns such as lipopolysaccharide and flagellin [7]. Epithelial cells, macrophages, and dendritic cells play an important role in the initial response to mucosal pathogens. However, there is mounting evidence that direct interaction of pathogens with these cells is not sufficient to generate an effective mucosal response.

Resident T cells represent a major component of the gut mucosa and are located in between epithelial cells (intraepithelial lymphocytes) and in the lamina propria [8]. It is the intraepithelial lymphocytes that likely contribute to the barrier function and integrity of the intestinal epithelium [9, 10]. Several subsets of T cells ($\alpha\beta$, $\gamma\delta$, NK, NK-T) are present in the gut mucosa and play an important role in mucosal immunity [11]. Perhaps the most striking evidence of the role T cells play in mucosal immunity comes from patients infected with the human immunodeficiency virus (HIV). HIV infects CD4⁺ T cells in the gut as early as a few weeks after infection, resulting in almost complete loss of this cell population [12–14]. Loss of mucosal CD4⁺ T-helper cells consequently results in a loss of function of both B cells and CD8⁺ T cells [15]. In HIV patients, CD4⁺ T cell depletion results in increased susceptibility to bacteremia caused by intestinal pathogens (mostly *Salmonella*, but also *Shigella* and *Campylobacter*) [16–18]. This indicates that CD4⁺ T cells are essential for the mucosal barrier against pathogens that normally cause inflammatory diarrhea.

In the mouse model, depletion of either CD4⁺ or CD8⁺ T cell subsets results in increased translocation of *E. coli* to the mesenteric lymph nodes [19]. While mice do not develop inflammatory diarrhea when infected with *S. typhimurium*, streptomycin-pretreated mice infected with *S. typhimurium* develop an acute inflammatory response in the cecum [20]. In this model, CD3⁺ (T cell) depletion results in dramatic reduction of the gross pathology, neutrophil influx, and expression of pro-inflammatory cytokines and chemokines [21]. Similarly, CD3⁺ depletion or the absence of $\alpha\beta$ T cells both result in reduced clearance of *C. rodentium* infection in mice [22, 23]. Overall, both clinical and experimental evidence reveal that mucosal T cells play an important role in containing commensals and pathogens to the gut.

Th17 Cells Orchestrate the Mucosal Response to Gut Pathogens

Several studies have suggested that a new subset of T cells, termed T-helper 17 (Th17) cells, orchestrate the mucosal defense against pathogens. Th17 cells constitute a distinct lineage from Th1 and Th2 cells and are characterized by the

production of a subset of cytokines: IL-17A, IL-17F, IL-22, and IL-26 [24]. Th17 cell differentiation is directed by the transcription factor ROR γ t, which is specific for the Th17 lineage [25]. The pro-inflammatory cytokines interleukin (IL)-6 and TGF- β appear to drive Th17 differentiation, at least in the mouse model [26], while the cytokine IL-23 appears to be indispensable for the protective effect of the Th17 response against mucosal pathogens like *C. rodentium*, *Klebsiella pneumoniae*, and *S. typhimurium* [27–29].

Another layer of complexity to the mucosal response to pathogens is that Th17 cytokines can be secreted by other cell types. IL-17 is released by $\gamma\delta$ T cells in response to IL-23 stimulation [30, 31]. NK and NK-T cells can produce IL-17 and IL-22 [32, 33]. Antigen-presenting cells such as dendritic cells can secrete IL-22 in response to bacterial infection [34]. The cytokines IL-17A, IL-17F, and IL-22 are expressed in the mucosa in response to several bacterial and fungal pathogens; examples include *K. pneumoniae* infection in the lung, *C. rodentium* and *S. typhimurium* infection in the gut, *Candida albicans* infection of the oral cavity, and many others (reviewed in [35]).

Adaptive T cells (Th17 cells), innate-like T cells ($\gamma\delta$ T cells), or both can contribute to the host mucosal immune response. The contribution of Th17 cells during *C. rodentium* infection is evident during the second week post-infection [27]. In contrast, IL-17 expression occurs quite early during *S. typhimurium* infection, ranging from 5 h post-inoculation of rhesus macaque ileal loops to 48 h post-oral infection in mice [29, 36]. The vast majority of gut T cells in the aforementioned rhesus macaque study were either CD4⁺ or CD8⁺ with $\gamma\delta$ T cells comprising less than 1% of the total gut T cell population; this result suggests that $\gamma\delta$ T cells are not a major source of IL-17 at 5 h post-*S. typhimurium* infection in adult rhesus macaques. In line with this observation, IL-17 production in response to *S. typhimurium* infection was dramatically decreased in macaques previously infected with the simian immunodeficiency virus which causes a selective loss of CD4⁺ T cells in the gut (comprising both Th1 and Th17 cells) [36]. In the mouse model, $\gamma\delta$ T cells contributed to the IL-17 produced at day 2 post-infection, but they were not the sole source [29]. It thus appears that early activation of both adaptive and innate-like T cells can lead to expression of IL-17 and IL-22. As these T cells express the IL-23 receptor, expression of IL-23 by dendritic cells comprises a common trigger or potentiating factor for early T cell activation prior to the development of adaptive immunity.

Th17 Development in the Gut Depends on Microbiota Composition

The gut microbiota is primarily composed of bacteria belonging to the phyla *Bacteroidetes* and *Firmicutes* [37,

38]. As demonstrated in gnotobiotic (lacking microbiota) mouse studies, development of gut immunity is strongly influenced by the intestinal microbiota. It was recently shown that some bacteria of the genus *Clostridia* (phylum *Firmicutes*), termed segmented filamentous bacteria (SFB), are essential to the development of Th17 cells in the mouse gut [39–42]. One host factor that influences the composition of the gut microbiota, including colonization by SFB, is the expression of antimicrobial peptides such as defensins [42]. Expression of a human specific alpha defensin (DEFA5) in the mouse dramatically changes the microbiota composition, including the loss of SFB [42]. Concomitant with these changes in the microbiota, fewer IL-17 producing T cells were isolated from the lamina propria. In contrast, mice colonized with SFB had an increased expression of genes associated with inflammation and antimicrobial defense and were subsequently more resistant to infection with *C. rodentium* [41]. These recent studies demonstrate that specific members of the gut microbiota can shape aspects of the mucosal barrier.

Th17 Cytokines Control Pathogen Dissemination from the Mucosa

The role IL-17 and IL-22 play during mucosal infections has become apparent following several studies that employed mice lacking these cytokines (IL-17A, IL-17F, IL-22), their receptors (IL-17Ra, IL-17Rc), or upstream cytokines responsible for their induction (IL-23 p19 deficient mice). During colonic infection with *C. rodentium*, IL-17A, IL-17F, and IL-22 appear to play a role in controlling the severity of gut pathology [34, 43]. In rhesus macaques, depletion of Th17 cells by SIV results in alteration of the mucosal barrier and increased dissemination of *S. typhimurium* to the mesenteric lymph nodes. Moreover, IL-17Ra^{-/-} mice have increased translocation of *S. typhimurium* to the mesenteric lymph nodes and spleen [36]. Ileitis caused by *Toxoplasma gondii* infection is dependent on IL-23 stimulated IL-22 production by CD4+ T cells in the small intestinal lamina propria [44]. As illustrated in these and other studies, IL-17 and IL-22 upregulation appear to comprise a stereotypic response to infection with mucosal pathogens.

Receptors of IL-17A and IL-17F (IL-17Ra and IL-17Rc) are expressed in several cell types, while receptors for IL-22 and IL-26 are expressed solely by epithelial cells [45–48]. Little is known about the role of IL-26 because this cytokine is not present in the mouse genome. In vitro stimulation of intestinal epithelial cells with IL-17, IL-22, or IL-26 induces changes in gene expression, including upregulation of chemokines (CXCL-8, CCL20) and antimicrobial responses (iNos, lipocalin-2) [49–52]. IL-17 also contributes to tight

junction formation and increased trans-epithelial resistance in polarized intestinal epithelial cells [53]. Both IL-17 and IL-22 stimulate granulopoiesis by inducing expression of the granulocyte colony stimulating factor G-CSF [54–58]. Induction of G-CSF and CXC chemokine expression contribute to neutrophil accumulation and function in the mucosa in response to infection [59, 60]. Th17 cytokines thus contribute to the mucosal barrier by several mechanisms which, upon activation, result in a mucosal immune response geared towards eliminating pathogens.

The mucosal response in patients with inflammatory diarrhea is characterized by a massive neutrophil infiltrate in the intestinal mucosa. Clinical evidence suggests that neutrophils are important in containing diarrheal pathogens to the gut and preventing their systemic spread. In patients affected by primary neutrophil immunodeficiency (i.e., chronic granulomatous disease), *S. typhimurium* often disseminates from the gut, resulting in bacteremia [61, 62]. HIV infection also results in a secondary neutrophil immunodeficiency [63, 64] which is likely a contributing factor to the susceptibility of HIV patients to bacteremia [65]. In the mouse model of inflammatory diarrhea, Th17 deficiency results in reduced neutrophil recruitment to the mucosa during infection with *S. typhimurium* [36]. Thus, a defect in neutrophil recruitment may explain why *S. typhimurium* dissemination increases in the absence of IL-17 signaling.

One of the mucosal responses induced during *C. rodentium* colonic infection is the secretion of antimicrobial C-type lectins of the RegIII family, including Reg3γ and Reg3β [34]. Induction of Reg3γ is dependent on IL-22 and is important in controlling intestinal infection with *C. rodentium* or vancomycin-resistant enterococci [34, 66]. β-defensin 1, 3, and 4 are also induced during *C. rodentium* infection in the gut by IL-17A and IL-17F [43], however the role these antimicrobial peptides play in this model is not yet known.

Gut Inflammation Promotes Pathogen Colonization

Intestinal pathogens replicate in the gut and reach high numbers in the stool in order to achieve transmission via the fecal–oral route. Replication in the gut requires adaptation to harsh conditions including the presence of bile salts, mucus, and the resident microbiota competing for nutrients and binding sites along the intestinal epithelium. Several recent studies suggest that pathogens have an advantage over the resident microbiota in colonizing the gut as both *C. rodentium* and *S. typhimurium* infections lead to overgrowth of the pathogen with concomitant growth suppression of the resident microbiota [67–70]. However, such suppression occurs only in the presence of an inflammatory response, so in the absence of inflammation, the microbiota constitute a barrier against *S. typhimurium* colonization [67, 68]. While

the mucosal response is thus effective in keeping pathogens confined to the gut mucosa, it has the unfortunate side effect of promoting pathogen overgrowth in the intestinal lumen.

The mechanisms by which the inflammatory response promotes pathogen growth are not completely understood. One hypothesis is that pathogens thrive in the inflamed gut because they have adapted to access nutrients during inflammation to a higher degree than the resident microbiota. One potential nutrient source during intestinal inflammation may be represented by mucins, which are upregulated by IL-17 and IL-22 stimulation of intestinal epithelial cells [49]. The ability of *S. typhimurium* to swim in the direction of nutrients increases the fitness of the organism in the inflamed intestine, but not in the normal gut [71]. Induction of the mucin MUC1 was observed in the distal colon of patients infected with *Salmonella saintpaul* and *Campylobacter jejuni* [72], suggesting that pathogen colonization of the mucus layer may be relevant for the pathogenesis of inflammatory diarrhea in humans. Fitting in with this idea, it was recently demonstrated that mucin can be degraded by a serine protease autotransporter (the Pic mucinase) expressed by enteroaggregative *E. coli* (EAEC) and *Shigella flexneri* [73, 74]. In a mouse model of infection, the Pic mucinase enhances EAEC colonization and growth in the presence of mucins, thereby enhancing fitness of this pathogen [74].

While the acquisition of carbon, nitrogen, and oxygen atoms are essential for the growth of any organism, so too is the acquisition metal ions. One host strategy to fight infections is to reduce the accessibility of metal ions. The most studied metal ion with regards to bacterial infection is iron, an essential micronutrient. Under normal conditions, iron is largely bound to serum transferrin. In the inflamed gut, lactoferrin secreted by neutrophils functions as another iron-binding molecule, exhibiting greater iron affinity at low Ph than transferrin [75]. To overcome these host defense mechanisms, bacteria secrete iron chelators known as siderophores which have a higher affinity for iron than either transferrin or lactoferrin [76, 77]. Once bound to iron, siderophores are internalized by specialized bacterial transport systems, thereby providing an iron source during iron starvation.

The siderophore enterochelin (produced by most members of *Enterobacteriaceae*), is a target of the host antimicrobial protein lipocalin-2 [78]. Lipocalin-2 binds to iron-laden enterochelin and prevents its uptake by bacteria, thereby inhibiting growth of susceptible strains [78, 79]. Both in vitro and in vivo, lipocalin-2 expression and secretion is dependent on stimulation of epithelial cells with IL-17 and IL-22 [49, 58]. Thus, lipocalin-2 expression is increased in mucosal surfaces, including the gut, during the course of mucosal infections. As an adaptation to this host defense, pathogens such as *S. typhimurium*, pathogenic *E. coli*, and some strains of *K. pneumoniae* have developed

resistance to lipocalin-2 by encoding for at least one additional siderophore, termed salmochelin [80–83]. Salmochelin is a glycosylated form of enterochelin that is not bound by lipocalin-2, thus its expression restores the ability of bacterial pathogens to acquire iron [49, 84–86].

Iron acquisition through salmochelin promotes *S. typhimurium* colonization of the inflamed gut, but not of the normal gut or in the absence of lipocalin-2 [49]. Thus, the capacity to acquire iron during intestinal inflammation is an important virulence trait for survival in the inflamed gut. Other mucosal pathogens have additional siderophores (yersiniabactin, aerobactin) which also provide an advantage for colonization of mucosal surfaces, although their role in iron acquisition in the inflamed gut remains to be determined [87–89]. Lipocalin-2 is also induced by pathogens at other mucosal surfaces including *Streptococcus pneumoniae* and *Haemophilus influenzae* in the nasal mucosa, *Helicobacter pylori* in the stomach, and *C. albicans* in the oral cavity [90–92]. These pathogens are not susceptible to lipocalin-2 mediated iron withholding because they do not acquire iron through enterochelin. Although its role in these infection models has yet to be established, it is plausible that lipocalin-2

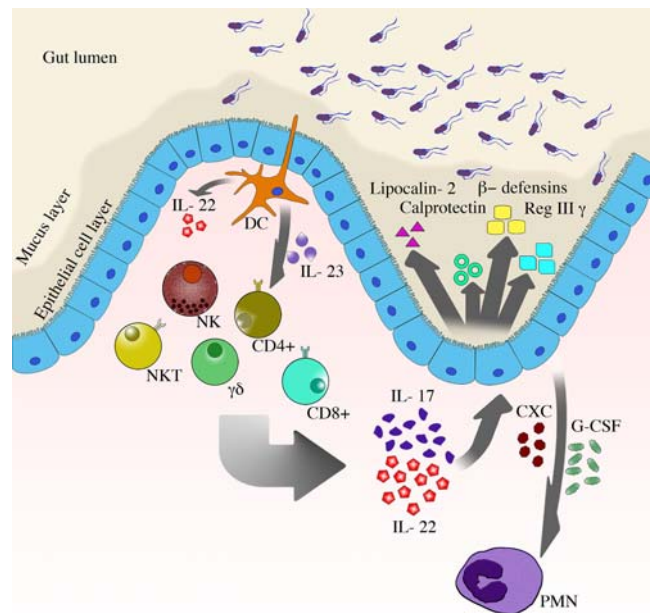


Fig. 1 Th17 cytokines and the gut mucosal barrier. Dendritic cells activated by pathogens secrete several cytokines including IL-22 and IL-23. IL-23 stimulates several subsets of T cells (Th17 cells, $\gamma\delta$ T cells, NK, and NK-T cells) to secrete IL-17 and IL-22. T cells promote amplification of the host response by stimulating the intestinal epithelium to secrete CXC chemokines (neutrophil chemoattractants) and antimicrobial peptides (lipocalin-2, calprotectin, Reg3 γ , and β -defensins). While the Th17 response prevents bacterial dissemination from the gut, it also promotes colonization of the mucosa by pathogens that are resistant to some of the induced antimicrobial responses. The ability to acquire nutrients and associate with the expanded mucous layer during inflammation promotes colonization of pathogenic microbes

production may be exploited by these pathogens to promote their colonization by suppressing the growth of competitors.

Other metal ions such as zinc and manganese are also essential micronutrients for bacteria. The antimicrobial peptide calprotectin, which is present in neutrophil granules, chelates zinc, and manganese, is involved in host defense against bacterial infections [93]. Expression of the S100A8 and S100A9 subunits of calprotectin is induced in the mucosa in response to IL-17 and IL-22 stimulation [34, 94]. The role that this and other antimicrobial peptides play in host defense to pathogens remains to be established.

Conclusions

The function of the cytokines IL-17, IL-22, and IL-23 in response to gut pathogens is summarized in the model proposed in Fig. 1. Dendritic cells activated by pathogens secrete cytokines including IL-22 and IL-23 [34, 95]. Several innate-like and adaptive T cells harbor the IL-23 receptor and are activated by this cytokine to release IL-17 and IL-22 [11]. These cytokines stimulate epithelial cells to express CXC chemokines resulting in neutrophil recruitment to the site of infection. Neutrophils constitute part of the mucosal barrier against inflammatory diarrheal pathogens by containing these infections to mucosal sites and preventing bacteremia [96].

IL-17 and IL-22 also induce the expression and secretion of antimicrobial peptides including lipocalin-2, Reg3 γ , β -defensins, and calprotectin [34, 43, 49, 58]. Some of these antimicrobial peptides may control dissemination from the mucosa, as in the case of Reg3 γ during *C. rodentium* infection [34], while others such as lipocalin-2 suppress the growth of commensal microbiota and consequently promote the growth of resistant pathogens [49]. Pathogens which have adapted to this hostile environment have acquired a range of virulence factors that provide access to nutrients in the inflamed gut, including glycoproteins in the mucus layer and metal ions.

Pathogens have thus evolved to take advantage of various aspects of the mucosal response to gain an edge over the resident microbiota in colonizing the inflamed gut. Even though the enhancement of pathogen colonization is a “side effect” of IL-17 and IL-22 mediated responses, resolution of diarrhea typically occurs within a few days of its onset. In contrast, defects in Th17-mediated responses like those observed in HIV patients result in increased dissemination of both pathogens and the microbiota, culminating in bacteremia with a high mortality rate [97, 98]. So while Th17 responses appear to be detrimental by promoting pathogen colonization of the mucosa, in the end it is the resulting decrease in bacterial dissemination from the mucosa that protects the host.

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