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Review

Immunomodulatory responses of the intestine to pathogenic bacterial colonization

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ABSTRACT

The human intestine is a biodiverse environment in which coordination, communication, and symbiotic relationships take place among hundreds of bacterial species and the host. Pathogenic bacteria may colonize the gut upon consumption of contaminated foods, and subsequent illness is often accompanied by a host inflammatory response. While a strong inflammatory response is initiated to battle infection, many bacteria simultaneously evade immune detection and killing as a survival strategy. This review highlights the interplay between the host induction of inflammation and the bacterial activation of anti-inflammatory signaling that occurs during pathogenic bacterial infection of the intestines. Pathogenic bacteria species covered here include *Salmonella enterica*, *Vibrio cholerae*, *Escherichia coli* and *Yersinia enterocolitica*. A thorough understanding of host immune responses as well as bacterial immune evasion strategies could provide more targets for future therapies.

KEYWORDS: inflammation, immune evasion, pathogenic bacteria, infection, intestine

INTRODUCTION

The human intestinal tract is colonized by as many as 10^{11} colony forming units per gram of bacteria to create a dense and diverse collection of microbes that fluctuate frequently [1]. To monitor this dynamic niche, an extensive network of host immune cells polices the gut. Symbiotic relationships between intestinal bacteria and host aid the human host in digestion, provide nutrients, and even assist in immunological tolerance [2, 3]. Though the digestive tract includes a wide array of microbiota distributed throughout the oral, esophageal, and gastric sections [1], this review will focus on bacteria of the intestines. The coevolution of host animals and bacteria has been ongoing long before the start of humans, and we are only beginning to understand the interconnection of our species with the microbiota that colonize it. Commensal bacteria interact with the host through secreted signals and sometimes by direct contact with the epithelial cell barrier. The host remains tolerant to commensal species due to low epithelial (Toll-like Receptor) TLR4, low co-activating molecule expression [4], and large abundance of regulatory T cells. Additionally, a mucosal barrier comprised of a tightly packed layer of mucus (20 μm (duodenum) to 100 μm (colon) thick) under a less dense layer (150 μm (duodenum) to 750 μm (colon) thick) [5] that contains mucin, antimicrobial molecules and proteins separates intestinal bacteria from the epithelial cells. This is the first line of host defense, with the second being the intestinal epithelial layer that when healthy is impenetrable to bacteria. A subset of epithelial cells that are specialized at sensing and regulating commensal and pathogenic bacteria to maintain overall homeostasis are Paneth cells [6]. These cells reside

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in intestinal crypts, or folds between villi, and secrete antimicrobial products to prevent the microbiota barrier breach. Overall, the epithelial layer is crucial for the allowance of commensal bacteria, but acts as an initial sensor and relays alarm signals to the immune system in the presence of pathogenic bacteria. The tolerance of commensal bacteria is balanced against activation of the immune system during damage and infection. This homeostatic balance is under constant fluctuation and regulation so that immune cells can be ready to eradicate invaders quickly.

Ingested pathogenic bacteria can infect mucosal tissues to cause gastroenteritis. During the process of infection, pathogens must penetrate several layers of host defense. Commensal bacteria are the first to encounter pathogenic bacteria in the intestines and can elicit "colonization resistance" to prevent colonization and growth of pathogenic invaders through limitation of available carbon sources and micronutrients, secretion of antimicrobials and toxins, and adhesion exclusion [7, 8]. Pathogenic bacteria can secrete mediators to combat these resident commensal species. After outcompeting the commensals, pathogenic bacteria must infiltrate the mucosal layer, and either invade or disrupt the epithelial barrier. They can accomplish this through secretion of mediators, such as toxins, virulence factors, or enzymes. Effectors may also be secreted directly from the bacteria into the cytoplasm of host cells through bacterial delivery mechanisms known as secretion systems. The type III secretion system (T3SS) is a common pathogenic tool for delivery of virulence factors from gram-negative bacteria into host cells. This complex consists of numerous organized peptides that form needle-like structures to puncture the mammalian cell membrane and deliver effectors directly to the cytoplasm. As reviewed by Mota and Cornelis, bacteria use the T3SS to inject effectors and subsequently control or modulate essential processes, such as phagocytosis, inflammatory signaling, apoptosis, autophagy, or intracellular trafficking [9]. The bacterial effectors that are delivered will determine the change in phenotype of the host cell.

Immune responses to pathogenic bacterial infection of the gut have been extensively reviewed [10, 11]. Briefly, immune cells, including epithelial cells, use pattern recognition receptors (PRRs) to detect microbial pathogen-associated molecular patterns (PAMPs). During such signaling, inflammatory pathways are triggered, including nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways. The classical NF-κB pathway is a hallmark pro-inflammatory pathway that converges numerous proinflammatory signals into the activation of one main heterodimeric transcription factor p65/p50. Activation of this robust transcription factor influences the transcription of hundreds of genes to participate in cell survival, inflammation, and immune cell activation. MAPK pathways are made up of several distinct or sometimes intertwined phosphorylation signaling cascades that culminate in the activation of various transcription factors that play roles in proliferation, differentiation and inflammation. Activation of either inflammatory pathway causes expression of activating surface receptors, cytokines, chemokines, and other signaling mediators that are subsequently released into the tissue to recruit and activate other immune cells. Commensal species avoid excessive proinflammatory signaling through a variety of mechanisms, such as keeping their distance from direct contact with the epithelial layer, or interfering with proinflammatory signaling pathways.

M cells are specialized epithelial cells that allow particulates and microbes to transcytose from the lumenal to basolateral side, where immune cells can then identify whether lumenal contents are harmful or not. Dendritic cells take up these transcytosed particulates, or they can also sample the lumenal environment through extension of pseudopodia through the epithelial layer. Next, these cells travel to lymph tissues to present the sampled antigens to T cells. If a T cell recognizes the presented antigen from an activated dendritic cell that is also delivering costimulatory signals, the T cell will become activated, clonally expand, and initiate an immunogenic response. Activated T cells can mount cytotoxic responses to infected cells, or activate B cells to secrete antibodies against pathogenic bacteria. The mesenteric lymph node is the major site of gut immunity, but other lymph node-like structures including Peyer's patches and inducible gut-associated lymphoid tissue (iGALT) line the intestines for local immune responses. These tissues are organized congregation

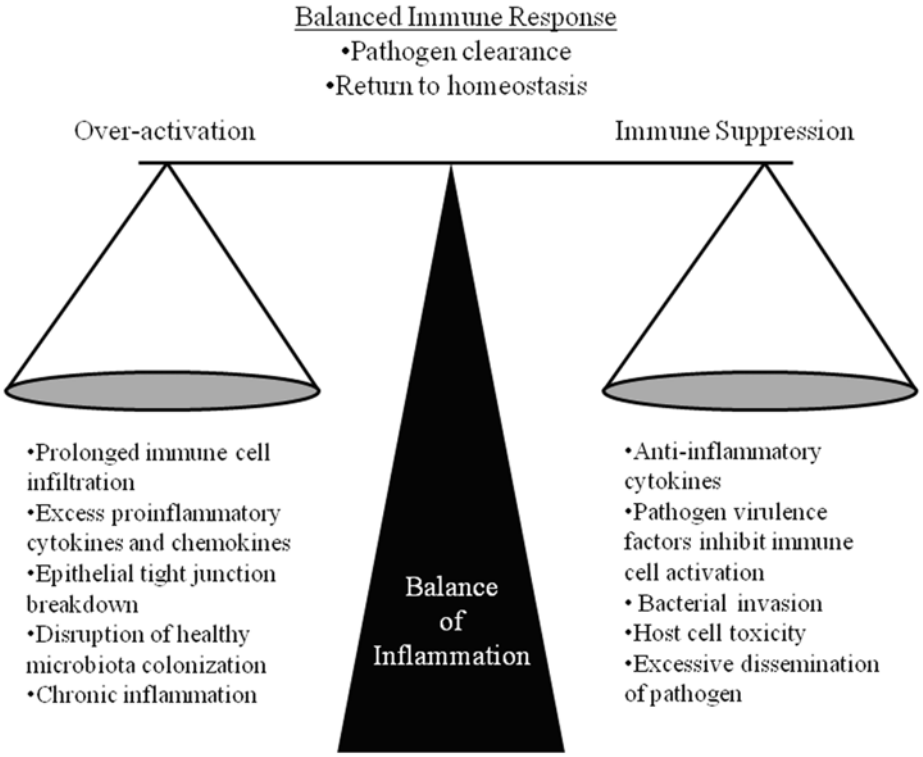

Figure 1. The balance of gut inflammation is tightly regulated. Immune responses to commensal microbrobes need to be regulated to allow for colonization and healthy homeostasis. However, after a pathogen challenge, immune responses need to defend against the harmful intruder. In doing so, resident immune cells recruit more cells to the inflamed tissue *via* cytokine production to mount a response against the pathogen. Cytokines are produced by all immune cell types to activate host cells to fight the infection. At the same time, pathogens are battling the host defenses with virulence factors and effectors. They act to suppress immunity in order to colonize and disseminate. For host survival to occur, the immune system must work quickly and effectively to eliminate the infection and return to homeostasis. Too much suppression caused by bacterial effectors could limit necessary immune responses and allow for increased infection. Too much inflammation may restrict pathogen infection but cause damage to the host tissue, possibly leading to prolonged chronic inflammation. Either extreme causes host morbidity exemplifying the delicate and tightly regulated balance of immune activation.

sites for immune cells to communicate and mount inflammatory immune responses when necessary. IgM and IgA, and upon infection, IgG are the main mucosal antibodies that can bind to gut microbiota, a process known as opsonization. Antibody-covered bacteria can prevent invasion of pathogenic bacteria into cells, or help deliver bacteria to phagocytic immune cells. Upon phagocytosis, bacteria can be killed in the lysosome, and their peptides can be presented on the surface for T cell activation. Commensal-specific IgA additionally acts to downregulate immunostimulatory bacterial epitope expression, thus dampening inflammatory responses against commensal bacteria, and adding to the tolerogenic environment [12].

Many cell types work in concert to maintain homeostasis of the gut. The tightly regulated "balance of inflammation" (Figure 1) exemplifies how a slight tipping of the balance in either direction can cause morbidity. For example, if immune cells are inhibited from activation, they would not be able to properly combat invading bacteria. Conversely, if immune cells overreact to commensal or other non-threatening signals, unnecessary inflammation would ensue, leading to chemokine and cytokine production, cell infiltration, edema, and tissue damage. Therefore, this balance

must remain steady to achieve homeostasis. This review focuses on mediators of pathogenic bacteria that alter normal pathways of inflammation as the host responds to the infection. In doing so, the pathogen benefits through further infiltration and/or immune-evasion. This manipulation of immune response can be achieved through 1. interference with and inhibition of pro-inflammatory pathways, 2. initiation of anti-inflammatory pathways through bacteria signals that mimic host ligands, or 3. disruption of the post-translational modifications involved in signaling. This review will discuss four species of pathogenic bacteria due to their interesting immunomodulatory strategies: *Salmonella enterica, Vibrio cholerae, Escherichia coli* and *Yersinia enterocolitica* or closely related pathotypes.

Salmonella enterica

Included in the *Salmonella enterica* species designation are several serovars that are the causative agents of disease in humans [13]. Infection by *S. Typhi* or *S. Paratyphi*, which manifests as systemic typhoid fever, is largely controlled in developed nations, yet remains endemic in developing nations [14]. In contrast, non-typhoidal *Salmonella* infections, for which *S. Typhimurium* and *S. Enteritidis* are the most common culprits, remain a global health concern. Agricultural control initiatives aimed at reducing *Salmonella* infection in the developed world have proven ineffective in curbing the infection rate. Indeed, in the United States the incidence of non-typhoidal *Salmonella* infection has risen from approximately 12 to 15 per 100,000 people over the period 1970-2013 [15].

For *S. Typhimurium* and *S. Enteritidis*, the major source of human disease is contaminated food products, ingestion of which results in intestinal infection and acute gastroenteritis. Though poultry meat and eggs are the best known sources of *Salmonella*, other types of meat and produce may also harbor *Salmonellae* [16]. The prevalence of these enteropathogens is attributable to their adaptability in host reservoirs, their resilience in various environments, and the complex control of virulence mechanisms that facilitates regulated induction and subversion of mammalian host defense.

Like other flagellated Gram-negative bacteria, the structural components of *Salmonella* spp. include PAMPs that induce an innate immune response in mammalian cells [17]. In addition to these classical activators of inflammatory signaling, *Salmonella* spp. also induce gastrointestinal inflammation by expressing *Salmonella* pathogenicity island-1 (SPI-1), which encodes the structural and effector components of a T3SS [18]. When expressed in the gut, the SPI-1 T3SS injects effectors into host epithelia that induce membrane ruffling and bacterial uptake [19]. A subset of these effectors (SopE, SopE2, SopB) are potent inducers of inflammation [20]. These proteins aid in bacterial uptake by activating Rho-GTPases, and simultaneously induce inflammation by activating MAPKs [21]. While host inflammation can be detrimental to bacteria, *Salmonella* has evolved a unique mechanism to benefit from inflammation in the gut. This mechanism is the use of tetrathionate (a byproduct of reactive oxygen species, which are produced during inflammation and neutrophil infiltration) as a terminal electron acceptor [22, 23]. The ability to reduce tetrathionate permits *Salmonella* to respire anaerobically in the gut, and so facilitates luminal proliferation. Thus, the result of injection of SPI-1 effectors into the epithelium is both bacterial invasion and pronounced tissue inflammation, which enables expansion of the luminal population of *Salmonella* [24]. To this end, *Salmonellae* enact a program of inflammation induction within the intestine [25]. However, once *Salmonellae* penetrate the gut epithelium and reside intracellularly in host macrophages, environmental signals trigger an altered epithelial cell gene expression program that represses intracellular immune processes to permit bacterial survival [26]. Thus, the spatial modulation of the host immune response, regulated by bacterial expression of dichotomous gene programs, enables *Salmonellae* to maximize their survival in various host niches.

Though inflammation in the intestinal lumen enhances *Salmonella* proliferation, these bacteria enact a program of immunosuppression once engulfed by host cells. This is achieved by the T3SS encoded within the *Salmonella* pathogenicity island-2 (SPI-2). SPI-2 induction upregulates effectors encoded within its own pathogenicity island, and additional coregulated effectors located elsewhere in the chromosome or on the *Salmonella* Virulence Plasmid [26]. Many of the upregulated

effectors target host processes to enable the formation of a *Salmonella*-containing vacuole *via* modulation of host cell cytoskeleton, vacuolar membrane maintenance, and intracellular trafficking modification [27-30]. Though differing in their specific targets, several SPI-2 effectors achieve the end result of inhibiting NF-κB activation. The plasmid-encoded effector SpvC acts upstream by dephosphorylating MAPKs, which in turn prevents NF-κB activation [31]. SspHI and SseL also prevent NF-κB activation. SseL specifically does so by deubiquitinating IκB, thereby preventing its dissociation from NF-κB. Downstream, SpvD prevents nuclear translocation of NF-κB by interfering with importin recycling [32]. Multiple modes of inflammatory signaling inhibition underscores the importance for the intracellular agenda of *Salmonella*.

SPI-2 effectors have also been shown to interfere with major histocompatibility complex (MHC) class II presentation of bacterial peptides [33]. This endpoint is achieved at two stages: first, a collection of effectors (SifA, SspH2, SlrP, PipB2, and SopD2) prevents peptide loading onto MHCII, thereby destabilizing unloaded MHC class II complexes [34], and second, loaded MHC class II complexes are ubiquitinated and impaired in their surface presentation [35]. The second mechanism is reliant on SPI-2, yet the implicated effector(s) remains unknown.

The cumulative result of immunosuppression is dampened induction of cytokines, reactive oxygen and nitrogen species, apoptosis, and pyroptosis. By inhibiting cellular immune response, *Salmonellae* create an environment that allows bacterial survival within the infected macrophage. While this stands in contrast to the inflammatory effectors of SPI-1, it is important to note that in each of these immunomodulatory programs there exist opposing effectors. For example, the SPI-1 encoded protein AvrA performs a function similar to SpvC by dephosphorylating MAPKs [36]. In fact, AvrA is secreted by both SPI-1 and SPI-2 [37]. Similarly, the SPI-2 effector SrfA has been shown to possess proinflammatory activity and its delivery to host cells activates NF-κB [38]. The presence of these contradictory effectors emphasizes the need for tightly regulated host immune modulation to optimize specific environments.

Vibrio cholerae

Several species in the *Vibrio* genus are enteric pathogens, including *V. parahemolyticus*, *V. vulnificus*, and *V. cholerae* [39]. Of these, *V. cholerae* is the best characterized. As *V. cholerae* is transmitted by the fecal-oral route, poor sanitation in developing countries contributes to prevalent infection [40]. When ingested, a number of host factors determine the severity of disease [41, 42]. The more severe manifestations of disease are referred to as cholera gravis, and are marked by gastroenteritis, severe diarrhea, and vomiting. Ensuing dehydration can be life-threatening [43].

V. cholerae possess a suite of virulence factors to trigger such pronounced symptoms, including toxins, pili, and virulence secretion systems [44, 45]. Amongst these, the eponymous cholera toxin (CT) is the best known. Intoxication by CT proceeds by a well-described mechanism, and is responsible for the massive intestinal water efflux that characterizes cholera gravis. An AB toxin, the CT holotoxin is comprised of a single catalytic A subunit (CTA) bound noncovalently to a homopentameric B subunit (CTB). The CTB pentamer binds to GM1 ganglioside receptors on the host cell and delivers the A subunit, which is responsible for host cell intoxication. The mechanism by which CTA acts is well defined, and is reviewed elsewhere [46]. In addition to the specific catalytic activity of CTA, host cells also exhibit altered immune function as a result of intoxication, characterized by enhanced production of prostaglandin, which exerts broad immunostimulatory effects [47].

While the A subunit is responsible for host cell intoxication, the CTB subunit is not inert. Studies have shown the CTB subunit to induce proinflammatory host responses. This response is induced when the CTB pentamer binds and crosslinks host cell GM1 ganglioside [48]. This inflammatory capacity of the CTB pentamer, along with its ability to deliver bound peptides, has made it a favorite vehicle in vaccine design. The numerous studies that have employed CTB as a delivery system by constructing alternate peptides to bind noncovalently to the CTB pentamer, taking the place of CTA, have also shown CTB to be a suitable adjuvant, augmenting host cell

response by virtue of its immunostimulatory capability [49, 50]. However, the activity of CTB is not so straightforward. Though CTB is itself proinflammatory, it also inhibits subsequent immune response to lipopolysaccharide (LPS) [51]. This complex effect was shown in macrophages pretreated with CTB, which dampens the subsequent respiratory burst upon LPS challenge. This effect is not unique to CTB, as additional components of *V. cholerae* impose a similar immunomodulatory effect. Among these are the outer membrane porins, or *omp*s, which can act as TLR1/2 ligands to induce host immune response [52]. Studies have demonstrated that, like CTB, purified OmpU induces an initial proinflammatory response in host cells, yet inhibits subsequent immune response to LPS [52, 53].

V. cholerae possess several additional virulence factors. One is the secreted toxin termed the large multifunctional repeat-in-toxin (MARTX) that carries out several functions *via* multiple effector domains, and represses host innate immune function. One effector domain is the alpha-beta hydrolase subunit. This esterase/lipase cleaves host cell phosphatidylinositol-3-phosphate, which ultimately impairs phagocytosis and aids bacterial survival in the gut [54].

Yet another tactic that *V. cholerae* employs is the perturbation of cell-mediated immunity by a type six secretion system (T6SS) that is activated in bacteria that have been phagocytosed by host immune cells. The effectors injected by the T6SS crosslink host cell actin, crippling further immune function [55]. For bacteria that enact this immunoevasion strategy, internalization by antigen-presenting cells is a dead-end, making this mechanism similar to suicidal altruism wherein phagocytosed bacteria sacrifice themselves to benefit the bystanders.

Another secretion system of *V. cholerae* is the T3SS that facilitates delivery of the virulence factor VopE. When injected into the host cell, VopE targets the host mitochondria and interacts with Miro, a Ras GTPase, by increasing its rate of GTP hydrolysis. This inactivates Miro, and in turn impairs mitochondrial participation in innate immune function [56]. The end result is diminished reactive oxygen species production, reduced NF-κB activation, and impaired immune sensing and clearance of *V. cholerae*.

The multitude of virulence factors employed by *V. cholerae* contributes to its ongoing prevalence. By dissecting the function of each of these components, individual effectors may be targeted to combat *Vibrio*'s pathogenicity, or exploited for their specific immunomodulatory activities.

Escherichia coli

Escherichia coli (*E. coli*) is a common microbe appearing in the microflora of many animals; however, with the right genes in the right environment, it can also be pathogenic. Six main pathotypes comprise enteric *E. coli* infection based on virulence factors, disease profile and phylogeny: 1. Enteropathogenic *E. coli* (EPEC), 2. Enterohamerrhagic *E. coli* (EHEC), 3. Enteroinvasive *E. coli* (EIEC), 4. Enteroaggregative *E. coli* (EAEC), 5. Enterotoxigenic *E. coli* (ETEC), and 6. Diffusely Adherent *E. coli* (DAEC). Two new groups have also been proposed, and all pathotypes are nicely reviewed by Clements *et al.* [57]. The United States Center for Disease Control and Prevention (CDC) identifies EHEC, also known as Shiga toxin-producing *E. coli*, as most commonly found in foodborne outbreaks, transmitted through contact with contaminated food, water, or animals. In 2013, there were 4,909 reported cases of culture-confirmed Shiga toxin-producing *E. coli* in the United States, and children between the ages of 1-4 had the highest incidence of infection among all age groups.

E. coli robustly trigger proinflammatory pathways through TLR signaling and delivery of specific mediators. For example, EspT causes expression and secretion of proinflammatory mediators such as KC, TNFα, IL-8, IL-1β and cyclooxygenase-2 (COX-2), and also signals Cdc42 and Rac1 to mediate bacterial invasion into epithelial cells [58]. Again, pathogenic triggering of inflammation initially aids in bacterial infection. To combat NF-κB-mediated inflammation, *E. coli* delivers effectors to decrease NF-κB signaling. Delivered from EPEC by a T3SS, NleE and NleB inhibit NF-κB signaling by blocking IκB degradation and therefore p65 subunit translocation into the nucleus [59]. Overall, NleE causes decreased IL-8, IL-1β, and both NleE and NleB decrease TNFα expression.

NleC uses zinc metalloprotease function to dampen NF-κB signaling through direct degradation of the NF-κB subunits p65, p50 and RelC to decrease IL-8 production [60]. NleD is another metalloprotease, which targets and cleaves c-Jun N-terminal kinases (JNKs) and p38 of the MAPK pathway [61]. Inhibition of these proinflammatory pathways decreases the immune response mounted against *E. coli*.

Pathogenic *E. coli* remains extracellular (except for EIEC) while colonizing the gut and invading the host. *E. coli* clearance is primarily mediated through opsonization followed by phagocytosis from professional phagocytes such as macrophages. Therefore, evasion or reduction of phagocytosis would be advantageous for bacterial survival. EspB suppresses phagocytosis through binding and inhibiting myosin interaction with actin filaments [62]. EPEC EspF reduces phosphatidylinositol 3-kinase (PI3K)-mediated phagocytosis [63]. EspJ is specifically able to block phagocytosis of opsonized bacteria [64]. EspH reduces both bacterial phagocytosis and FcγR-mediated phagocytosis of opsonized bacteria through blockade of Rho GTPase signaling [65]. Pinheiro da Silva *et al*. found that *E. coli* induces FcRγ phosphorylation and SHP-1 recruitment after binding a bacterial ligand to FcγRIII (CD16) [66]. Their group recently identified *wzxE* as a candidate gene that is responsible for this binding interaction [67]. CD16 signaling ultimately impaired macrophage receptor with collagenous structure (MARCO)-mediated phagocytosis of *E. coli* while increasing reactive oxygen species (ROS) and TNFα production [66, 67]. Decreased bacterial clearance would increase *E. coli* dissemination within the host.

Pathogens often affect host cell health through disruption of mitosis or apoptosis. The EPEC and EHEC cycle inhibiting factor (Cif) arrests eukaryotic cell cycle in the G2 phase, thus blocking mitosis [68]. The enzyme is so valuable for pathogen infection that its essential catalytic triad is conserved in homologous proteins across species, with the closest homolog from *Yersinia pseudotuberculosis* [69]. Cytolethal distending toxins also blocks the G2-M transition, likely through DNase activity [70]. Independent of its aforementioned function in blocking phagocytosis, EspF is another inducer of apoptosis. EspF interferes with the membrane potential of the mitochondria to induce the release of cytochrome c, ultimately initiating apoptosis with subsequent caspase 9 and 3 cleavage [71]. Yet another known function of EspF is its ability to increase epithelial permeability through redistribution of the tight junction protein occludin [72]. Inhibiting cell proliferation or causing apoptosis would also have profound negative impacts on intestinal epithelial maintenance and integrity, and could greatly restrict the expansion of responding lymphocytes.

In addition to secreting soluble effectors through the T3SS, EPEC inserts an immunoreceptor tyrosine-based inhibitory motif (ITIM)-bearing membrane protein, Tir into the epithelial cell membrane. This is a classic example of host protein mimicry, in which pathogens influence host cell signaling using bacterial proteins to carry out mammalian signals. ITIM is a short conserved peptide displayed on the cytoplasmic side of immune cell receptors that generally inhibits the activation of signaling pathways. Once implanted in the host cell membrane, the extracellular portion of Tir binds to the bacterial protein intimin, providing bacterial adhesion and signaling from the outside of the cell. This *de novo* signaling in the mammalian cell suppresses TRAF-6-mediated proinflammatory cytokine production [73, 74]. Tir also mediates the rearrangement of actin filaments beneath the host cell membrane through direct interaction with Nck, to form the characteristic pedestal complex of infected cells [75]. This multifaceted protein greatly contributes to the immune evasion of *E. coli*.

E. coli exploits inflammation to initiate infection, and subsequently combats immune response with effectors that signal and suppress it. Survival of the host is then determined by the power of the immune response against infection.

Yersinia enterocolitica

Yersinia enterocolitica is a Gram-negative rod-shaped bacteria with six major biotypes, most of which are pathogenic, providing the ability to invade mammalian cells. *Y. enterocolitica* mainly causes gastrointestinal disease, but depending on the route of infection and spread after infection, it can result in septicemia, metastatic infections, pharyngitis, reactive arthritis, and erythema nodosum.

The CDC estimates that *Y. enterocolitica* causes almost 117,000 illnesses, 640 hospitalizations, and 35 deaths in the United States every year, and affects countries worldwide. Swine are the main carriers of these bacteria, but depending on the serotype reservoirs, may include farm animals, wild mammals, pets, waterfowl, surface water and sewage. Following ingestion of contaminated food, *Y. enterocolitica* migrates through the mucous layer and preferentially binds to M cells to be taken up where they gain access to intestinal tissues [76].

Yersinia virulence originates from the plasmid for *Yersinia* virulence (pYV) and some chromosome-encoded factors. *Yersinia* outer proteins (Yops) are potent virulence effectors, whose genes are located on pYV. These effectors are essential to *Yersinia* infection and are delivered to eukaryotic cells through their T3SS. Yops act through mimicry or enzymatic activity to interfere with host cellular pathways. The Yops highlighted here have roles in immune evasion. Some have been more thoroughly studied in other *Yersinia* species, but still have relevance to known or unknown orthologs of *Y. enterocolitica* pathogenicity. The ability of *Yersinia* virulence factors to influence immune responses is hardly new. In 1990, the YopE protein was shown to potently inhibit pathogen phagocytosis by macrophages, and without it, oral and intraperitoneal administrations of the bacteria are avirulent in animal infection models [77].

Disruption of intracellular signaling is a powerful offense against the immune system. Effector protein YopM of *Yersinia pestis* has been shown to mimic eukaryotic phosphatases that target and mute inflammatory signaling of caspase 1, which becomes activated to restrict bacterial survival in the host [78]. YopE and YopH are other phosphatases of *Yersinia* spp., as identified through homology studies [79]. Indeed, YopH of *Yersinia pseudotuberculosis* is a tyrosine phosphatase that was shown to affect phosphorylation of two inflammatory pathways to decrease neutrophil activation and ultimately increase bacterial survival [80]. YopH also inhibited calcium responses *via* dephosphorylation of SH2 domain-containing leukocyte protein of 76 kDa (SLP-76) in isolated neutrophils [80]. YopH can directly inhibit T cell responses by targeting adaptor proteins Linker for Activated T cells (LAT) and SLP-76 of the T cell receptor signaling pathway [81]. YopH was shown to inactivate the PI3K pathway in macrophages and T cells. In macrophages, this resulted in decreased production of the cytokine monocyte chemoattractant protein-1 (MCP-1). In T cells, the inhibition of PI3K resulted in decreased IL-2 production and proliferation [82].

A soluble protein from *Y. enterocolitica*, low calcium response V antigen (LcrV), can be released into invaded tissues to cause immunosuppression. Specifically, it decreases TNFα secretion from activated macrophages through the induction of the anti-inflammatory cytokine IL-10, thereby redirecting the programming of the macrophages from proinflammatory to anti-inflammatory [83].

YopJ (and possibly its ortholog in *Y. enterocolitica*, YopP) has recently been declared an acetyltransferase [84], although this remains somewhat controversial. Addition of an acetyl group on critical phosphorylation residues blocks the potential of host cell phosphorylation at that given residue. Therefore, important proinflammatory signal cascades are disrupted. For example, YopJ has been shown to block phosphorylation of critical residues of MAPKK6 and hence, inhibits MAPK, NF-κB, and likely, other signaling pathways [84]. Combined inhibition of both MAPK and NF-κB signaling pathways causes apoptosis of macrophages [85], quenching the potential inflammatory signaling from a key immune cell. Yop P/J inhibits JNK and p38 activation in dendritic cells resulting in less antigen uptake, less pathogenic antigen presentation to T cells, and ultimately, decreased adaptive immune response against the pathogen [86]. YopJ also may inhibit TLR3-mediated interferon regulatory factor-3 (IRF3) signaling as shown through inhibition of polyI:C-activated, TLR3-expressing HEK293 cells [87]. While specific signaling mechanisms are not yet known, YopB is an effector shown to decrease TNFα production in Peyer's patches and macrophages [88]. Ultimately, decreases in cytokine production result in decreased immune cell activation and recruitment, giving the pathogen the ability to continue its infection.

Aforementioned *Yersinia* Cif-like homolog Ypk1971 enacts cell cycle arrest and actin cytoskeleton

rearrangement [69]. YopO, is a serine threonine kinase that has been recently crystallized [89]. Its activation also causes disruption in the actin cytoskeleton. To protect itself from host cell phosphatases, it has multiple residues for autophosphorylation to maintain its activity. Disruption of the cytoskeleton is a common means of attack across many pathogens, indicating the success of this offense. It could lead to inhibition of phagocytosis to prevent bacterial clearance, inhibition of motility, disruption of mitosis, and changes in important morphology.

In summary, *Yersinia* can combat host immune signaling through secreted and outer protein signaling. For further information, Pha and Navarro, and Dhar and Virdi, have recently and comprehensively reviewed these and other *Yersinia* effectors [90, 91].

CONCLUSION

The presence of pathogenic bacteria triggers numerous pro-inflammatory pathways in their hosts. Pathogens capitalize on certain initial inflammatory processes to aid in invasion. However, excess sustained inflammation would eliminate bacterial survival and growth. Therefore, the ability of pathogens to control host inflammation is essential to their continued existence. In this review, we highlight evidence of bacteria employing signaling molecules and effectors to modulate pro-inflammatory immune signaling. The existence of such signaling supports centuries of evolution to allow bacteria to survive by evading gut immunity. We did not cover metabolic changes induced by pathogens, which also play important roles in modifying the overall immune response.

From a drug therapy standpoint, immunogenic isolates of intestinal pathogenic bacteria may be exploited to intentionally manipulate host responses. Immunogenic (proinflammatory) bacteria have been evaluated as vaccine delivery agents, with the intention that an inflammatory host response to the bacterial delivery agent might increase the host immune response to a transgenically expressed antigen. Thus, the immunogenic delivery strain acts as an adjuvant or an agent to increase immune response and immune memory against the antigen it is expressing.

Conversely, immunosuppressive bacteria could be of therapeutic use for prevention of unwanted inflammation. For example, orally inoculated pretreatment of virulence-attenuated *Yersinia pseudotuberculosis* was found to reduce colonic lesions and TNFα levels of an induced colitis mouse model [92]. Therefore, individual strains of bacteria may be employed to achieve an intended host immune status, with the resulting immune state reflective of the cumulative response to a myriad of signaling inputs generated by the bacteria.

More effective than this broad immune manipulation is the targeted manipulation of specific host signaling pathways by bacterial effectors. In this approach, individual bacterial effectors can be exploited for their immunomodulatory properties, exclusive of the confounding effects of additional bacterial components. This approach also enables directed modulation of selected signaling pathways implicated in specific disease states. Furthermore, since individual effectors can be delivered or expressed from a delivery strain of choice, this approach avoids potential complications that arise from bacterial vectors that grow poorly in the gut, or that fail to elicit the desired immune response.

The concert of bacterial *vs*. host signaling has been playing for millions of years, and constantly adapting to each other. A deeper investigation into the interplay of bacteria effectors with host inflammatory signaling can help us understand the pathogenesis of bacteria, design specific treatments for infections, and usurp natural signaling events for new therapies.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Tlaskalova-Hogenova, H., Stepankova, R., Kozakova, H., Hudcovic, T., Vannucci, L., Tuckova, L., Rossmann, P., Hrncir, T., Kverka, M., Zakostelska, Z., Klimešová, K.,

Přibylová, J., Bártová, J., Sanchez, D., Fundová, P., Borovská, D., Srůtková, D., Zídek, Z., Schwarzer, M., Drastich, P. and Funda, D. P. 2011, Cell Mol. Immunol., 8(2), 110-120.
2. Cario, E. 2005, Gut, 54(8), 1182-1193.
3. Mowat, A. M. 2003, Nat. Rev. Immunol., 3(4), 331-341.
4. Abreu, M. T., Fukata, M. and Arditi, M. 2005, J. Immunol., 174(8), 4453-4460.
5. Atuma, C., Strugala, V., Allen, A. and Holm, L. 2001, Am. J. Physiol. Gastrointest. Liver Physiol., 280(5), G922-G929.
6. Vaishnava, S., Behrendt, C. L., Ismail, A. S., Eckmann, L. and Hooper, L. V. 2008, Proc. Natl. Acad. Sci. USA, 105(52), 20858-20863.
7. Sassone-Corsi, M. and Raffatellu, M. 2015, J. Immunol., 194(9), 4081-4087.
8. Stecher, B. and Hardt, W. D. 2008, Trends Microbiol., 16(3), 107-114.
9. Mota, L. J. and Cornelis, G. R. 2005, Ann. Med., 37(4), 234-249.
10. Perez-Lopez, A., Behnsen, J., Nuccio, S. P. and Raffatellu, M. 2016, Nat. Rev. Immunol., 16(3), 135-148.
11. Pédron, T. and Sansonetti, P. 2008, Cell Host Microbe, 3(6), 344-347.
12. Peterson, D. A., McNulty, N. P., Guruge, J. L. and Gordon, J. I. 2007, Cell Host Microbe, 2(5), 328-339.
13. US Department of Health and Human Services, CDC. 2013, An Atlas of Salmonella in the United States, 1968-2011: Laboratory-based Enteric Disease Surveillance, Atlanta, GA.
14. Crump, J. A. and Mintz, E. D. 2010, Clin. Infect. Dis., 50(2), 241-246.
15. US Department of Health and Human Services, CDC. 2016, National Salmonella Surveillance Annual Report, 2013, Altanta, GA.
16. United States Department of Agriculture. 2011, Salmonella Questions and Answers.
17. Keestra-Gounder, A. M., Tsolis, R. M. and Baumler, A. J. 2015, Nat. Rev. Microbiol., 13(4), 206-216.
18. Srikanth, C. V., Mercado-Lubo, R., Hallstrom, K. and McCormick, B. A. 2011, Cell Mol. Life Sci., 68(22), 3687-3697.
19. Zhou, D., Chen, L. M., Hernandez, L., Shears, S. B. and Galan, J. E. 2001, Molecular Microbiology, 39(2), 248-259.
20. Patel, S. and McCormick, B. A. 2014, Front. Immunol., 5, 311.
21. Hobbie, S., Chen, L. M., Davis, R. J. and Galan, J. E. 1997, J. Immunol., 159(11), 5550-5559.
22. Winter, S. E., Thiennimitr, P., Winter, M. G., Butler, B. P., Huseby, D. L., Crawford, R. W., Russell, J. M., Bevins, C. L., Adams, L. G., Tsolis, R. M., Roth, J. R. and Bäumler, A. J. 2010, Nature, 467(7314), 426-429.
23. Thiennimitr, P., Winter, S. E., Winter, M. G., Xavier, M. N., Tolstikov, V., Huseby, D. L., Sterzenbach, T., Tsolis, R. M., Roth, J. R. and Baumler, A. J. 2011, Proc. Natl. Acad. Sci. USA, 108(42), 17480-17485.
24. Chirullo, B., Pesciaroli, M., Drumo, R., Ruggeri, J., Razzuoli, E., Pistoia, C., Petrucci, P., Martinelli, N., Cucco, L., Moscati, L., Amadori, M., Magistrali, C. F., Alborali, G. L. and Pasquali, P. 2015, Front. Microbiol., 6, 985.
25. Rivera-Chavez, F. and Baumler, A. J. 2015, Annu. Rev. Microbiol., 69, 31-48.
26. Figueira, R., Watson, K. G., Holden, D. W. and Helaine, S. 2013, MBio, 4(2), e00065.
27. Freeman, J. A., Ohl, M. E. and Miller, S. I. 2003, Infection and Immunity, 71(1), 418-427.
28. Beuzón, C. R., Méresse, S., Unsworth, K. E., Ruíz-Albert, J., Garvis, S., Waterman, S. R., Ryder, T. A., Boucrot, E. and Holden, D. W. 2000, EMBO J., 3(19), 15.
29. Ohlson, M. B., Fluhr, K., Birmingham, C. L., Brumell, J. H. and Miller, S. I. 2005, Infect. Immun., 73(10), 6249-6259.
30. Yu, X. J., Liu, M. and Holden, D. W. 2016, MBio, 7(4), pii: e00474-16.
31. Mazurkiewicz, P., Thomas, J., Thompson, J. A., Liu, M., Arbibe, L., Sansonetti, P. and Holden, D. W. 2008, Mol. Microbiol., 67(6), 1371-1383.
32. Rolhion, N., Furniss, R. C., Grabe, G., Ryan, A., Liu, M., Matthews, S. A. and Holden, D. W. 2016, PLoS Pathog., 12(5), e1005653.

33. Tobar, J. A., Carreno, L. J., Bueno, S. M., Gonzalez, P. A., Mora, J. E., Quezada, S. A. and Kalergis, A. M. 2006, Infect. Immun., 74(11), 6438-6448.
34. Halici, S., Zenk, S. F., Jantsch, J. and Hensel, M. 2008, Infect. Immun., 76(11), 4924-4933.
35. Lapaque, N., Hutchinson, J. L., Jones, D. C., Meresse, S., Holden, D. W., Trowsdale, J. and Kelly, A. P. 2009, Proc. Natl. Acad. Sci. USA, 106(33), 14052-14057.
36. Jones, R. M., Wu, H., Wentworth, C., Luo, L., Collier-Hyams, L. and Neish, A. S. 2008, Cell Host Microbe, 3(4), 233-244.
37. Giacomodonato, M. N., Noto Llana, M., Aya Castaneda Mdel, R., Buzzola, F. R., Sarnacki, S. H. and Cerquetti, M. C. 2014, Microbiology, 160(Pt 6), 1191-1199.
38. Lei, L., Wang, W., Xia, C. and Liu, F. 2016, J. Immunol., 196(2), 792-802.
39. Centers for Diseasee Control and Prevention. 2016, National Enteric Disease Surveillance: COVIS Annual Summary, 2014, Atlanta, GA.
40. Organization, W. H. 2014, Weekly Epidemiological Record, 89, 12.
41. Harris, J. B., Khan, A. I., LaRocque, R. C., Dorer, D. J., Chowdhury, F., Faruque, A. S., Sack, D. A., Ryan, E. T., Qadri, F. and Calderwood, S. B. 2005, Infect. Immun., 73(11), 7422-7427.
42. Mosley, W. H., Ahmad, S., Benenson, A. S. and Ahmed, A. 1968, Bull World Health Organ., 38(5), 9.
43. Daniels, N. A. and Shafaie, A. 2000, Infect. Med., 17(10), 665-685.
44. Withey, J. H., Nag, D., Plecha, S. C., Sinha, R. and Koley, H. 2015, Antimicrob. Agents Chemother., 59(12), 7471-7476.
45. Bina, X. R., Taylor, D. L., Vikram, A., Ante, V. M. and Bina, J. E. 2013, MBio, 4(5), e00366-13.
46. Vanden Broeck, D., Horvath, C. and De Wolf, M. J. 2007, Int. J. Biochem. Cell Biol., 39(10), 5.
47. Duebbert, I. E. and Peterson, J. W. 1985, Toxicon, 23(1), 16.
48. Connell, T. D. 2007, Expert Rev. Vaccines, 6(5), 821-834.
49. Jani, D., Singh, N. K., Bhattacharya, S., Meena, L. S., Singh, Y., Upadhyay, S. N., Sharma, A. K. and Tyagi, A. K. 2004, Plant Cell Rep., 22(7), 471-477.
50. Russell, M. W., Wu, H-Y., Hajishengallis, G., Hollingshead, S. K. and Michalek, S. M. 1999, Advances in Veterinary Medicine, 41, 10.
51. Rodríguez, B. L., Rojas, A. and Benítez, J. A. 1998, FEMS Immunol. Med. Microbiol., 22(3), 6.
52. Sakharwade, S. C., Sharma, P. K. and Mukhopadhaya, A. 2013, PLoS One, 8(9), e76583.
53. Sakharwade, S. C. and Mukhopadhaya, A. 2015, Mol. Immunol., 68(2 Pt A), 312-324.
54. Agarwal, S., Kim, H., Chan, R. B., Agarwal, S., Williamson, R., Cho, W., Paolo, G. D. and Satchell, K. J. 2015, Nat. Commun., 6, 8745.
55. Satchell, K. J. 2009, Cell Host Microbe, 5(3), 213-214.
56. Suzuki, M., Danilchanka, O. and Mekalanos, J. J. 2014, Cell Host Microbe, 16(5), 581-591.
57. Clements, A., Young, J. C., Constantinou, N. and Frankel, G. 2012, Gut Microbes, 3(2), 71-87.
58. Raymond, B., Crepin, V. F., Collins, J. W. and Frankel, G. 2011, Cell Microbiol., 13(12), 1881-1893.
59. Newton, H. J., Pearson, J. S., Badea, L., Kelly, M., Lucas, M., Holloway, G., Wagstaff, K. M., Dunstone, M. A., Sloan, J., Whisstock, J. C., Kaper, J. B., Robins-Browne, R. M., Jans, D. A., Frankel, G., Phillips, A. D., Coulson, B. S. and Hartland, E. L. 2010, PLoS Pathog., 6(5), e1000898.
60. Pearson, J. S., Riedmaier, P., Marchès, O., Frankel, G. and Hartland, E. L. 2011, Mol. Microbiol., 80(1), 219-230.
61. Baruch, K., Gur-Arie, L., Nadler, C., Koby, S., Yerushalmi, G., Ben-Neriah, Y., Yogev, O., Shaulian, E., Guttman, C., Zarivach, R. and Rosenshine, I. 2011, EMBO J., 30(1), 221-231.
62. Iizumi, Y., Sagara, H., Kabe, Y., Azuma, M., Kume, K., Ogawa, M., Nagai, T., Gillespie, P. G., Sasakawa, C. and Handa, H. 2007, Cell Host Microbe, 2(6), 383-392.

63. Quitard, S., Dean, P., Maresca, M. and Kenny, B. 2006, Cell Microbiol., 8(6), 972-981.
64. Marchès, O., Covarelli, V., Dahan, S., Cougoule, C., Bhatta, P., Frankel, G. and Caron, E. 2008, Cell Microbiol., 10(5), 1104-1115.
65. Dong, N., Liu, L. and Shao, F. 2010, EMBO J., 29(8), 1363-1376.
66. Pinheiro da Silva, F., Aloulou, M., Skurnik, D., Benhamou, M., Andremont, A., Velasco, I. T., Chiamolera, M., Verbeek, J. S., Launay, P. and Monteiro, R. C. 2007, Nat. Med., 13(11), 1368-1374.
67. Beppler, J., Mkaddem, S. B., Michaloski, J., Honorato, R. V., Velasco, I. T., de Oliveira, P. S., Giordano, R. J., Monteiro, R. C. and Pinheiro da Silva, F. 2016, Eur. J. Immunol., 46(8), 1926-1935.
68. Marchès, O., Ledger, T. N., Boury, M., Ohara, M., Tu, X., Goffaux, F., Mainil, J., Rosenshine, I., Sugai, M., De Rycke, J. and Oswald, E. 2003, Mol. Microbiol., 50(5), 1553-1567.
69. Jubelin, G., Chavez, C. V., Taieb, F., Banfield, M. J., Samba-Louaka, A., Nobe, R., Nougayrède, J. P., Zumbihl, R., Givaudan, A., Escoubas, J. M. and Oswald, E. 2009, PLoS One, 4(3), e4855.
70. De Rycke, J. and Oswald, E. 2001, FEMS Microbiol. Lett., 203(2), 141-148.
71. Nougayrède, J. P. and Donnenberg, M. S. 2004, Cell Microbiol., 6(11), 1097-1111.
72. McNamara, B. P., Koutsouris, A., O'Connell, C. B., Nougayréde, J. P., Donnenberg, M. S. and Hecht, G. J. 2001, Clin. Invest., 107(5), 621-629.
73. van Avondt, K., van Sorge, N. M., Sorge, N. M. and Meyaard, L. 2015, PLoS Pathog., 11(3), e1004644.
74. Yan, D., Quan, H., Wang, L., Liu, F., Liu, H., Chen, J., Cao, X. and Ge, B. 2013, Cell Signal., 25(9), 1887-1894.
75. Gruenheid, S., DeVinney, R., Bladt, F., Goosney, D., Gelkop, S., Gish, G. D., Pawson, T. and Finlay, B. B. 2001, Nat. Cell Biol., 3(9), 856-859.
76. Bottone, E. J. 1999, Microbes Infect., 1(4), 323-333.
77. Rosqvist, R., Forsberg, A., Rimpiläinen, M., Bergman, T. and Wolf-Watz, H. 1990, Mol. Microbiol., 4(4), 657-667.
78. LaRock, C. N. and Cookson, B. T. 2012, Cell Host Microbe, 12(6), 799-805.
79. Kaniga, K., Uralil, J., Bliska, J. B. and Galán, J. E. 1996, Mol. Microbiol., 21(3), 633-641.
80. Rolán, H. G., Durand, E. A. and Mecsas, J. 2013, Cell Host Microbe, 14(3), 306-317.
81. Gerke, C., Falkow, S. and Chien, Y. H. 2005, J. Exp. Med., 201(3), 361-371.
82. Sauvonnet, N., Lambermont, I., van der Bruggen, P. and Cornelis, G. R. 2002, Mol. Microbiol., 45(3), 805-815.
83. Sing, A., Roggenkamp, A., Geiger, A. M. and Heesemann, J. 2002, J. Immunol., 168(3), 1315-1321.
84. Mukherjee, S., Keitany, G., Li, Y., Wang, Y., Ball, H. L., Goldsmith, E. J. and Orth, K. 2006, Science, 312(5777), 1211-1214.
85. Zhang, Y., Ting, A. T., Marcu, K. B. and Bliska, J. B. 2005, J. Immunol., 174(12), 7939-7949.
86. Autenrieth, S. E., Soldanova, I., Rösemann, R., Gunst, D., Zahir, N., Kracht, M., Ruckdeschel, K., Wagner, H., Borgmann, S. and Autenrieth, I. B. 2007, Cell Microbiol., 9(2), 425-437.
87. Sweet, C. R., Conlon, J., Golenbock, D. T., Goguen, J. and Silverman, N. 2007, Cell Microbiol., 9(11), 2700-2715.
88. Beuscher, H. U., Rödel, F., Forsberg, A. and Röllinghoff, M. 1995, Infect. Immun., 63(4), 1270-1277.
89. Lee, W. L., Grimes, J. M. and Robinson, R. C. 2015, Nat. Struct. Mol. Biol., 22(3), 248-255.
90. Pha, K. and Navarro, L. 2016, World J. Biol. Chem., 7(1), 1-13.
91. Dhar, M. S. and Virdi, J. S. 2014, Microbes Infect., 16(2), 87-95.
92. Marceau, M., Dubuquoy, L., Caucheteux-Rousseaux, C., Foligne, B., Desreumaux, P. and Simonet, M. 2004, Infect. Immun., 72(4), 2438-2441.