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The role of chemical signaling and gut mucosal immunity during *Citrobacter rodentium* infection

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"*If you can dream it, you can do it*."

Walt Disney

Abstract

The human pathogens enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) are transmitted by the fecal-oral route and cause severe diseases worldwide. Due to their potential to induce pathological lesions in the gut epithelium, they are commonly known as *attaching and effacing* pathogens (A/E pathogens). To date, effective strategies to manage EPEC and EHEC infections beyond standard supportive measures are lacking. Therefore, preclinical studies to better understand the mechanisms of infection of EPEC and EHEC are required. Because these bacteria are poorly pathogenic in mice, *Citrobacter rodentium* has been broadly used as an alternative *in vivo* model pathogen to study EHEC and EPEC disease. Similarly, to human pathogens, *C. rodentium* causes A/E lesions in mice without requiring antibiotic pretreatment to establish infection.

Studies have shown that the membrane-bound histidine sensor kinase QseC promotes A/E pathogen colonization *in vivo*. However, whether QseC signaling is essential for A/E pathogens to resist and evade immune responses to establish disease remains unknown. On the host side, interleukin IL-22 is critical to resolve *C. rodentium*-mediated disease, although the exact mechanism(s) of IL-22-mediated protection are not well understood. Here, we aimed to investigate the role of chemical signaling through the QseC sensor for establishing disease and resistance to the host immune response. Furthermore, we studied the downstream mechanisms that mediate the mucosal immune protection conferred by IL-22 during *C. rodentium* infection.

Our data suggest that the QseC sensor is directly involved in triggering intestinal inflammation and promoting transmissible colonic crypt hyperplasia, a hallmark pathological feature of *C. rodentium*-mediated disease. Moreover, QseC-mediated signaling enhanced *C. rodentium*'s resistance to neutrophil antimicrobial responses, resulting in the pathogen's systemic dissemination *in vivo*. We identified changes in the lipopolysaccharide membrane modulated by the QseC sensor as a potential mechanism. Furthermore, we found that the IL-22-mediated resolution of *C. rodentium* infection was independent of the induction of several downstream responses, including calprotectin, Reg3β, Reg3γ, lipocalin-2, and C3. Instead, the susceptibility of IL-22 deficient mice to *C. rodentium* was primarily associated with increased intestinal permeability. Together, our data show that *C. rodentium* depends on chemical signaling mediated by QseC for disease establishment and to resist the immune responses conferred by neutrophils. Upon infection, IL-22 plays an essential role in host protection, which was independent of a single deficiency of either antimicrobial peptide regulated by this cytokine; instead, protection was related to IL-22's regenerative properties by promoting the epithelial

barrier integrity. Overall, our work expands the knowledge of the *in vivo* mechanisms triggered by A/E pathogens during infection.

Keywords: Chemical Signaling, Citrobacter rodentium, Immune response, Interleukin-22.

Resumo

Escherichia coli enteropatogênica (EPEC) e *E. coli* enterohemorrágica (EHEC) são patógenos humanos transmitidos pela via fecal-oral que causam doenças graves mundialmente. Devido a lesão que desenvolvem em células epiteliais intestinais, essas bactérias são conhecidas como patógenos *Attaching and Effacing* (patógenos A/E). Até o momento nenhuma estratégia eficaz foi desenvolvida para combater as infecções causadas por EHEC e EPEC, sendo o tratamento restrito apenas a suporte. Portanto, estudos pré-clínicos para melhor entender os mecanismos de infecção de EPEC e EHEC são necessários. Como essas bactérias são pouco patogênicas em camundongos, *Citrobacter rodentium* tem sido utilizada como um patógeno modelo alternativo para estudar *in vivo* a doença causada por EHEC e EPEC, uma vez que causa lesão A/E em camundongos e não requer pré-tratamento com antibióticos para estabelecer a infecção.

Estudos demonstraram que o sensor QseC, presente na membrana de bactérias, beneficia a colonização de patógenos A/E em vivo. No entanto, pouco se sabe se a sinalização através do sensor QseC em patógenos A/E é essencial para evadir e resistir às respostas imunes do hospedeiro para o estabelecimento da doença. Ainda, estudos demonstram que a interleucina-22 é essencial para a resolução da doença causada por *C. rodentium* em camundongos embora o(s) mecanismo(s) exato(s) de proteção mediada por Il-22 ainda necessitem de mais investigação. Desta forma, este trabalho foi desenvolvido com o intuito de investigar a participação da sinalização química através do sensor QseC para estabelecimento da infecção e resistência à resposta imune do hospedeiro, e o mecanismo mediado por IL-22 que confere proteção durante a infecção por *C. rodentium.*

Nossos resultados sugerem que o sensor QseC está diretamente envolvido no desencadeamento da inflamação intestinal e desenvolvimento da hiperplasia transmissível da cripta colônica, sendo esta última, uma característica da doença mediada por *C. rodentium*. Além disso, através da sinalização mediada por QseC, *C. rodentium* tem sua sobrevivência a neutrófilos aumentada, o que pode estar associado com sua disseminação sistêmica *in vivo* que pode ser devido a mudanças na membrana de lipopolissacarídeo modulada pelo sensor QseC. Ainda, nossos resultados sugerem que uma vez estabelecida a infecção, a citocina IL-22 é fundamental para a resolução da doença mediada por *C. rodentium*, uma vez que camundongos deficientes em IL-22 não sobrevivem à infecção. Porém esse fenótipo não é dependente de respostas mediadas pelas proteínas antimicrobianas: calprotectina, REG3, lipocalina-2 e C3, mas pode estar associada à indução de mecanismos de regeneração e integridade do epitélio mediados por IL-22. Juntos, esses resultados demonstram que patógenos A/E

dependem da sinalização química via QseC para resistir à resposta imune mediada por neutrófilos e para o estabelecimento da doença causada por *C. rodentium* em camundongos. Após a infecção, IL-22 desempenha um papel essencial na proteção do hospedeiro, que não depende da resposta isolada de peptídeo antimicrobianos regulados por esta citocina; ao contrário, essa proteção pode estar associada à recuperação do epitélio intestinal mediada por IL-22, promovendo integridade epitelial. De modo geral, nosso trabalho amplia o conhecimento dos mecanismos *in vivo* desencadeados por patógenos A/E durante a infecção.

Palavras-chave: Sinalização química, *Citrobacter rodentium*, Resposta Imune, Interleucina-22

Summary

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List of Abbreviations

CXCR2 - *C-X-C Motif Chemokine Receptor 2*

CXCL - *C-X-C Motif Chemokine Ligand*

IL-22 - *Interleukine-22*

Lcn2 - *Lipocalin-2*

REG3 - Islet-derived protein 3

S100A9 - S100 calcium-binding protein

C3 - Complement component 3

NET - *Neutrophil extracellular traps*

% - Percentage

A/E - *Attaching and Effacing*

AI-3 - Autoinducer-3

C. rodentium - Citrobacter rodentium

TCCH - *Transmissible colonic crypt hyperplasia*

CFU - *Colony Forming Unit*

FITC - *Fluorescein isothiocyanate*

OD600 - *Optical density at 600 nm*

EHEC - *Enterohaemorrhagic Escherichia coli*

EPEC - *Enteropathogenic Escherichia coli*

LB - *Lysogeny broth*

LEE - *Locus of Enterocyte Effacement*

LPS - *Lipopolysaccharide*

MOI - *Multiplicity of Infection*

QseC - *Quorum sensing E. coli C (sensor kinase)*

HUS - *Hemolytic uremic syndrome*

STEC - *Shiga Toxin-Producing Escherichia coli*

S. Typhimurium - *Salmonella enterica* serovar Typhimurium

T3SS - *Type III Secretion System*

qRT-PCR - *Quantitative Reverse Transcriptase Polymerase Chain Reaction*

Chapter I: Literature Review

1. Introduction

1.1. The gastrointestinal tract

The gastrointestinal tract (GI tract) is part of the digestive system of humans and other animals that extends from the mouth to the anus and contains the esophagus, stomach, and small and large intestines (1). The gut is a muscular tube composed of the luminal epithelium and the underlying lamina propria and muscularis mucosae layers. In addition to the GI tract, the digestive system also comprises the accessory organs: tongue, salivary glands, pancreas, liver, and gallbladder. Its primary function is the digestion and absorption of food (1,2).

The GI tract is colonized by a collection of bacteria, archaea, viruses, and fungi termed “gut microbiota” (3,4). The gut microbiota is a microbial community estimated to exceed 10^{14} microorganisms that co-evolved to form a mutually beneficial relationship with their host (3,5). The microbial composition differs between hosts and is often influenced by health, age, gender, and diet differences (4). The gut microbiota is fundamental to the host’s health. Its beneficial roles include digesting food and harvesting energy, training and regulating host immunity, strengthening gut integrity, and protecting against pathogens (3,5).

The microbiota is compartmentalized within the host, and its interactions are tightly regulated by multiple layers of physical, chemical, and immunological barriers (6); the intestinal epithelium is a single layer segregating the outer world from the inner body (7,8). It is covered by the mucus layer, which comprises mucins, antimicrobial proteins, and

Immunoglobulin (Ig) A, thereby preventing microorganisms from reaching the epithelial cells (9–12). The IEC monolayer is composed of specialized cell types that differentiate between residing epithelial cells, goblet cells, absorptive enterocytes, enteroendocrine cells, Microfold cells, and Paneth cells (13,14). These cells confer functions that range from nutrient absorption to mucus and antimicrobial peptide production for host protection (14). The underlying lamina propria consists of connective tissue with interspersed immune cells, separating the epithelial layer from the smooth muscle (15,16). The numerous immune cells within this layer have an essential role in homeostasis maintenance and defense against external threats (**Figure 1**). Moreover, Peyer's patches, the draining mesenteric lymph nodes (9,17), which, along with the diffuse lymphoid tissue of the lamina propria, form the gut-associated lymphoid tissue (GALT) that fundamentally contributes to the host's health by conferring protection and tolerance from foreign antigens and microbes alike due to the immune cells that habits this lymphoid tissues (9,17,18).

The dynamic between the host, environmental factors such as diet, and microbiota are complex. Clinical trials in humans have been conducted to understand this dynamic. However, there are limitations in the studies due to practical and ethical reasons. For these matters, mouse models have been extensively used to understand the interaction between intestinal microbiota, gut immune response, and diet within the gastrointestinal tract (19).

1.2. Gut immunity: a fine balance between protection and tolerance

The immune system constitutes a complex network of biological processes that involve protective responses against threats within an organism. Traditionally, the

immune system has been classified into two subsystems: innate and adaptative immunity, although with overlaps. The innate immune response has a limited recognition of molecular patterns. In contrast, the adaptive immune system mounts specific responses by recognizing a wide range repertoire of molecules (20).

The intestinal mucosa is continuously exposed to a variety of environmental signals. The gut immune system is challenged to balance homeostasis and distinguish commensal microorganisms from pathogenic bacteria, which is essential for eliminating pathogens and maintaining tolerance towards the microbiota and dietary antigens (15). The immune system associated with the intestinal mucosal is mainly located in the GALT (21). The latter is organized in the diffuse lymphoid tissue of the intestinal lamina propria, the intraepithelial lymphocyte compartment, and organized lymphoid follicles, such as Peyer's Patches (15,21). Some examples of immune cells that are involved in host protection within the intestinal lamina propria include monocytes, macrophages, neutrophils, dendritic cells (DCs), T and B lymphocytes, mast cells, and eosinophils **(Figure 1)** (15).

Microorganisms can be recognized by intra- and extracellular pattern-recognition receptors (PRRs) of immune cells and epithelial cells, which detect the pathogen-associated molecular patterns (PAMPs) or microbial-associated molecular patterns (MAMPs) from microorganisms. In turn, PAMPs and MAMPs are essential and well-preserved microorganism components that microbes cannot modify easily and are absent from the host (22–24). Among PRRs, detecting signals through cell membrane-bound Toll-like receptors (TLR) by mucosal tissue-resident cells is the first step of a protective host response (22). The engagement of TLRs with microbial PAMPs induces the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ)

(22). These events lead to cell activation and trigger the release of cytokines, such as Interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-1β, and surface costimulatory molecules for antigen presentation in tissue-resident antigen-present cells (APCs), such as macrophage and dendritic cells (22,25). Moreover, the release of chemokines and cytokines during infections of the gut mucosa prompts the migration of immune cells like monocytes and neutrophils from the peripheral blood to the site of infection (26). Neutrophils have a particularly critical role in protecting from and controlling infections and will be discussed in detail later in this literature review.

The pro-inflammatory environment during infections induces the maturation and migration of APCs, especially dendritic cells, to mesenteric lymph nodes through afferent lymphatic vessels, thereby regulating the adaptative immune responses (27). The priming of naïve T-lymphocytes through APCs leads to the differentiation and clonal expansion of effector T helper (Th, identified by CD4+ expression) cells and the subsequent activation of B cells for antibody assembly (27). The CD4+ effector T cells exit the mesenteric lymph node and migrate to the site of infection. In the gut, CD4+ T cells are essential in coordinating an adequate response for pathogen control and clearance (28).

T cells are classically subdivided according to the pattern of cytokines produced upon activation (15). Lamina propria CD4+ T helper cells are essential for resolving a variety of infections. Among T helper cells, Th1 cells mainly produce IL-2 and interferon (IFN)-γ and are usually involved in responses against intracellular pathogens (29). Th2 cells predominantly produce IL-4, IL-5, and IL-13 and are critical for response against helminths (15,29). Th17 cells typically produce IL-17 and IL-22 cytokines, and their activation mainly occurs upon exposure to IL-23 cytokine release by APC. Th17 cell

responses play essential roles in protection against bacterial and fungal infections (15,30).

Among the various cytokines produced in the gut, the interleukin IL-22 is a cytokine that plays a crucial role in gut protection. It is released mainly by Type 3 innate lymphoid cells (ILC3s) and T CD4+ cells upon stimulation of proinflammatory signals, such as IL-23, produced by myeloid cells (31). ILC3s are part of the innate immune response that is mainly described as resident-tissue cells in peripheral tissues that are able to respond promptly via the production of cytokines, such as IL-22, due to their location and effector phenotype that does not require priming to expand and activate (32–34). Upon infection, ILC3s are an important early source of IL-22 (33). On the other hand, Th22 and Th17 are between the main populations of the adaptative response responsible for producing and maintaining the levels of IL-22 upon a tissue perturbation by infection and/or inflammation (35–37). In the gut, the cytokine IL-22 is critically involved in different aspects of the mucosal defense, ranging from epithelial barrier homeostasis to the production of mucus and antimicrobial peptides to control and resolve inflammation (38).

During infection, the production of IL-22 stimulates the release of antimicrobial proteins by epithelial and immune cells, such as lipocalin-2, calprotectin, and regenerating islet-derived protein 3-beta and -gamma (Reg3β and Reg3γ) (38–42). Lipocalin-2 binds to bacterial siderophores, which are small molecules essential for bacteria to scavenge iron (43,44). Calprotectin chelates metals, including manganese and zinc (45). In turn, the restriction of metal acquisition by these proteins is a crucial mechanism for controlling the expansion of pathogens during infection (43). Also, Reg3 proteins exerts direct antibacterial activity by binding to components of bacterial membranes such as peptidoglycan (46) and lipid A (47).

Although protection against hazards is essential for the host to survive, immune cells are challenged in distinguishing between self and potential threats to the host once a directed response against the body's antigens can be detrimental. The gut immune tolerance within the gastrointestinal (GI) tract prevents the body from initiating harmful or exaggerated immune responses against antigens found in the gut, such as food particles and commensal bacteria (48,49). Some examples of key mechanisms of gut immune tolerance include the actions of regulatory T cells (T regs), immune cells in the GALT, the mucosal barrier, and the gut microbiota (48). T regs can induce tolerance by expressing immune suppressive cytokines such as TGF−β and IL-10 (50–52) or by limiting the proliferation of cells (51,53,54). Some types of GALT-associated DCs, for example, have been shown to present tolerogenic and immunoregulatory responses, thus inducing T regs cells (55–57). The physical epithelial barrier, in addition to the production of mucus and antimicrobial proteins, reduces the excessive contact between immune cells and antigens (58). Finally, the microbiota, through different mechanisms, helps to train the immune response (49,56). Together, these mechanisms educate the immune system to distinguish between harmful pathogens and benign antigens, promoting a balanced and tolerant immune response within the gut, which is crucial for maintaining gut health and preventing unnecessary inflammation and autoimmune reactions within the digestive system (56).

Although the microbiota is not part of the mucosal immune system per se, it is the first barrier pathogens encounter within the host. The microorganisms inhabiting the host prevent pathogens from gaining access to nutrients, a phenomenon termed “colonization resistance” (3,59). This is well supported by studies in germ-free mice and antibiotic-treated mice, which develop more severe infections with enteric pathogens than mice with

a normal microbiota (60–62). The microbiota also prevents the overgrowth of pathogens through signals that stimulate host immunity to induce the expression of antimicrobial peptides by Paneth cells and epithelial cells (9).

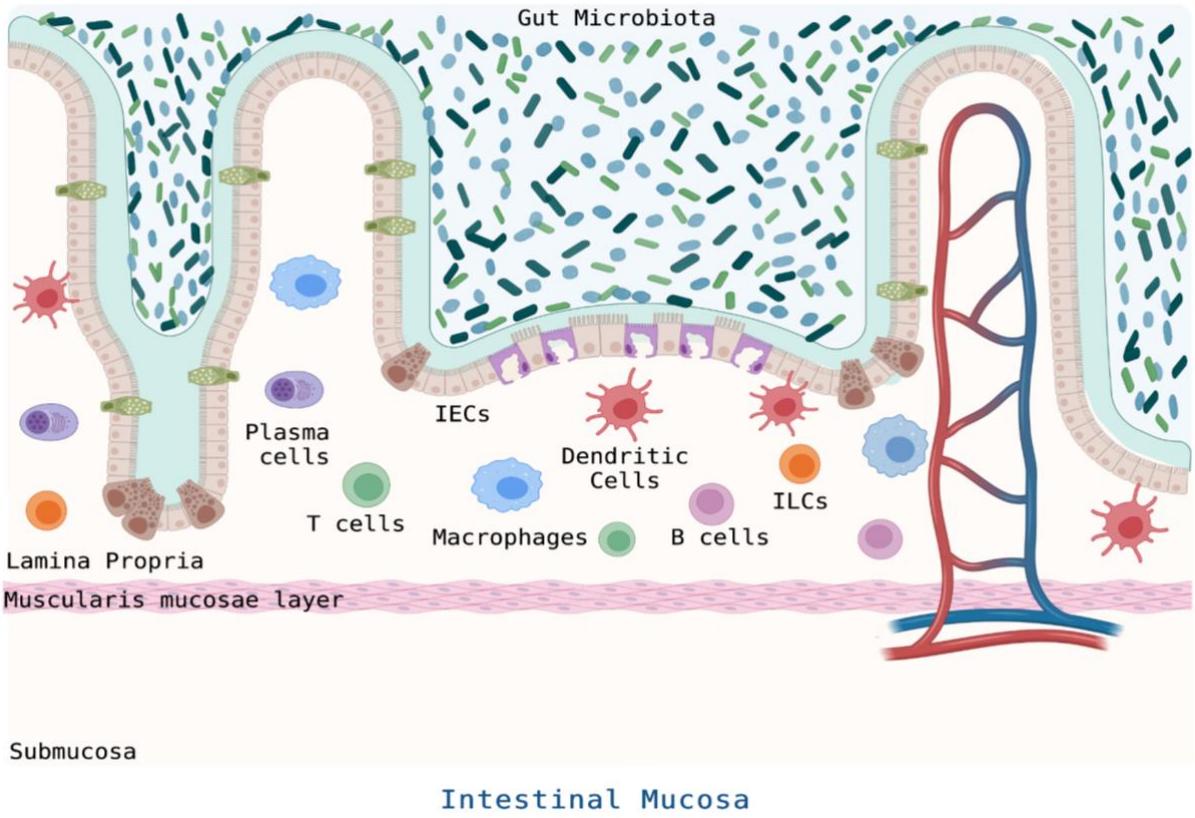

Figure 1. General Schematic of the intestinal mucosa. The gut is a muscular tube consisting of the luminal epithelium, underlying lamina propria, and muscularis mucosae layers. The luminal epithelium consists of a single layer of Intestinal epithelial cells (IECs) and specialized cell types such as goblet cells, enterocytes, enteroendocrine cells, Microfold cells, and Paneth cells. The lamina propria is a connective tissue layer with various immune cells such as B cells, dendritic cells, innate lymphoid cells (ILCs), T cells, plasma cells, and macrophages, among others. This layer separates the epithelium from the muscularis mucosae, and this organization is essential for the gut's physiological functions (Figure created with Biorender).

1.3. Neutrophils and host protection

Neutrophils are the most abundant phagocytic cell type of the innate immune system and constitute the first line of defense against infection. Neutrophils are derived from self-renewing hematopoietic stem cells in the bone marrow, which is regulated by

the cytokine granulocyte monocyte colony-stimulating factor (GM-CSF) (63). Mature neutrophils are retained in the bone marrow by expression of surface C-X-C Motif Chemokine Receptor 4 (CXCR4) and high constitutive production of its ligand C-X-C Motif Chemokine Ligand 12 (CXCL12) from osteoblast cells (64,65). Mature neutrophils are constantly released into the peripheral blood, which is greatly enhanced upon inflammatory stimuli or infection, resulting in more rapid mobilization of circulating neutrophils (66). The granulocyte colony-stimulating factor (G-CSF) promotes neutrophil exit from bone marrow by interfering with CXCR4-CXCL12 interaction (67). Besides, the chemokine receptor CXCR2 facilitates the egress of neutrophils from the bone marrow due to the constitutive production of its ligands CXCL1 and CXCL2 by endothelial cells (68).

During inflammation, tissue-resident cells such as macrophages and mast cells release chemoattractants and proinflammatory mediators (e.g., CXCL1, IL-1β, and TNF), which in turn facilitate the recruitment of neutrophils from the peripheral blood into the inflamed tissue (65). Adherence to the endothelium is initially mediated by a loose interaction between the neutrophil P-selectin glycoprotein ligand 1 (PSGL-1) and the endothelial E and P-selectins. Next, a firm adhesion is mainly mediated by the high affinity interaction of the integrin LAF-1 (leukocyte function-associated antigen 1) on neutrophils with ICAM1 (intracellular adhesion receptor 1) on endothelial cells (65,67). Binding promotes the vascular adhesion of neutrophils and, consequently, their translocation into inflamed tissue (65). In addition, increased expression of the CXCR2 ligands, CXCL1 and CXCL2, is involved in guiding neutrophils to the injured tissue (69).

Neutrophils are essential in controlling infection due to their high quantity of intracellularly stored granules that contain various proinflammatory and antimicrobial

proteins (70). At the site of infection, recognition of pathogens through NOD-like receptors (NLRs) and TRLs on the surface of neutrophils induce responses that include intracellular activation, degranulation, phagocytosis, and release of neutrophil extracellular traps (NETs) **(Figure 2)** (71). After phagocytosis, neutrophils eliminate pathogens via the production of reactive oxygen species (ROS) in the phagosomes and by producing and releasing antimicrobial peptides such as myeloperoxidase, elastase, cathepsins, defensins, lactoferrin, and lysozyme (70,71).

Another mechanism of neutrophils that contributes to immune protection is the release of neutrophil extracellular traps (NETs) (72) in a process known as NETosis. NETs can be formed in two ways; one way is driven by the cell death pathway that begins with nuclear delobulation and dismantlement of the nuclear envelope until complete abrogation of cellular polarization, followed by chromatin decondensation and plasma membrane rupture. The alternative route is known as non-lytic NETosis, which occurs independently of cell death and is related to the secreted expulsion of nuclear chromatin followed by the release of granule protein (71). Although an excessive release of NETs can be detrimental to the host's health, studies have shown that NETs have an essential role in the host's defense against pathogenic microorganisms (71,73–75).

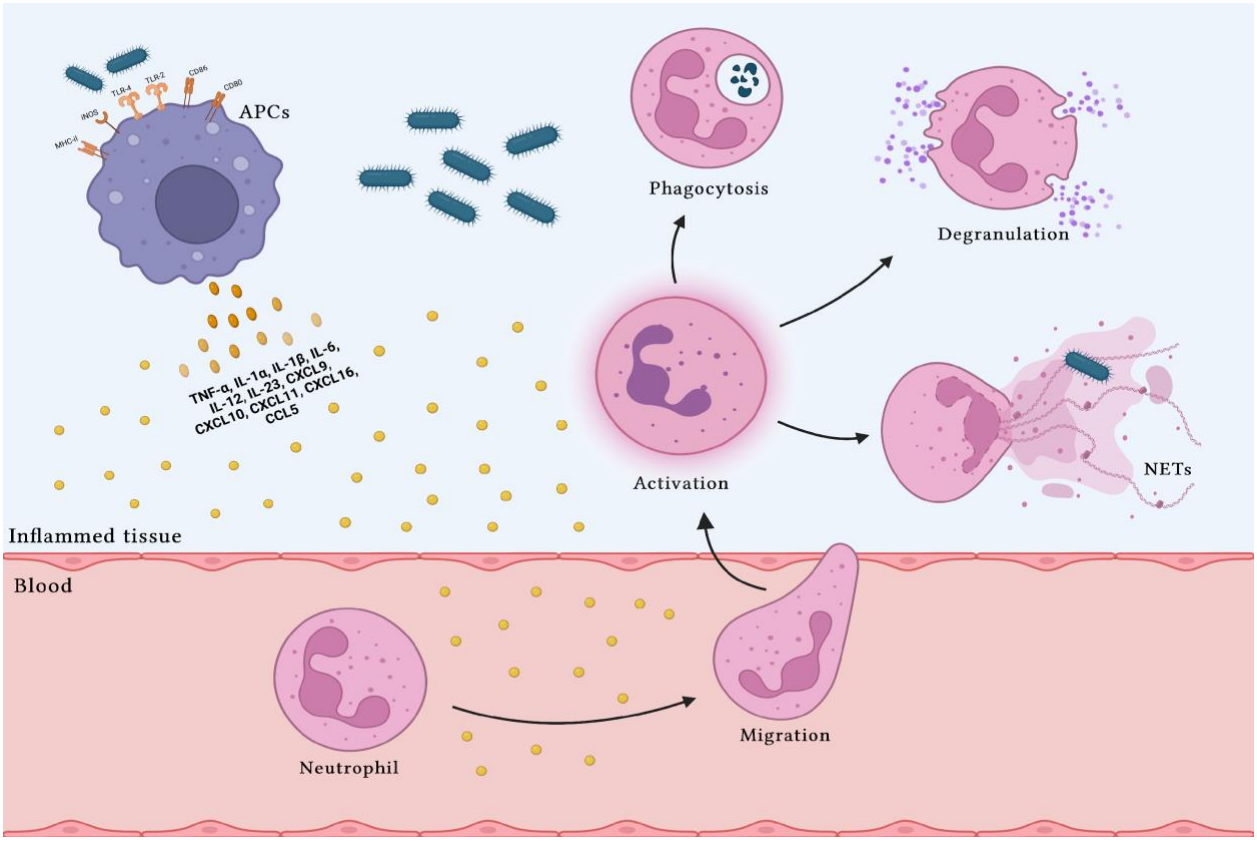

Figure 2. Neutrophils mediate responses at the site of infection. The figure elucidates the intricate process of neutrophil response to a stimulus triggered by a microorganism. Upon the production of cytokines and chemokines by tissue-resident antigen-presenting cells, neutrophils migrate from the blood to the injured tissue, where they become activated by the local environment. Upon activation, neutrophils engage in pathogen elimination through phagocytosis, respiratory burst, degranulation, and NETosis (Figure created with Biorender).

1.4. Pathogenic *Escherichia coli*: EHEC and EPEC

The human gut microbiome is a complex community composed of 10^{13}-10^{14} microorganisms and forms a mutualistic relationship with their host (3,76). Among the various genera that compose the human microbiota, the genus *Escherichia* is a widely distributed group inhabiting the large intestine of humans and other warm-blooded species (77,78). The Gram-negative bacterium *Escherichia coli* (*E. coli*) belongs to the *Escherichia* genus and represents a harmless commensal that frequently colonizes the human gut and rarely causes disease. However, throughout evolution, some clones have

acquired virulence genes that can cause severe illness in healthy individuals, therefore being designated pathogenic *Escherichia coli* (79).

Due to the genetic diversity of pathogenic *E. coli*, diseases caused by this microorganism are manifold. They can range from gastrointestinal tract infections, mainly assigned to enteric pathogenic *E. coli*, to extraintestinal infections involving sites such as the urinary tract, bloodstream, and central nervous system (80). Enteric pathogenic *E. coli* is usually restricted to the gastrointestinal tract and is classified according to the primary mechanism of virulence. So far, seven categories of enteric pathogens have been described: enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), adherent-invasive *E. coli* (AIEC), diffusely adherent *E. coli* (DAEC), enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) (79,81,82).

The diarrheagenic EHEC and EPEC are human pathogens transmitted by the fecal-oral route and are primarily associated with consuming contaminated food and water (81). EHEC is a subset of Shiga toxin-producing *E. coli* (STEC), well-known for causing hemorrhagic colitis, followed by the potential development of Hemolytic Uremic Syndrome (HUS) in healthy individuals (79). HUS is characterized by the triad of thrombocytopenia, mechanical hemolytic anemia, and acute kidney injury (83) and more often affects susceptible patient groups, including infants, children, and the elderly (83,84). EHEC outbreaks have a high prevalence in developed countries, although developing countries such as Argentina have presented the highest HUS incidence in children under the age of five (80). Antibiotic treatment is not recommended for STECs due to the enhancement of Shiga-toxin release and increased development of UHS (85). Treatment is supportive and includes rehydration and, in some cases, dialysis (86).

Diarrhea caused by EPEC has been associated with severe disease and mortality in children worldwide. Although EPEC infections in developed countries have largely disappeared, they remain a significant public health concern in undeveloped countries (80) mainly for causing persistent and potentially fatal diarrhea in children under two years old (82,87).

Due to the histopathological lesions on intestinal epithelial cells, known as *Attaching* and *Effacement* lesions (A/E lesions), EHEC and EPEC are known as *Attaching and Effacing* pathogens (A/E pathogens). The ability to induce A/E lesions is conferred by a group of virulence genes denominated Locus of Enterocyte Effacement Island (LEE Island) (88). The LEE Island is a 35.6Kb pathogenicity island in their genome consisting of 41 genes organized into five operons called LEE1, LEE2, LEE3, LEE4, and LEE5 (88–90). Once activated, the LEE genes encode structural components of the type III secretion system (T3SS), regulators, chaperones, and effector proteins (90). Through the T3SS system, which resembles a needle-like structure, A/E pathogens inject effector proteins and the receptor Tir into the host cell (91). Tir binds to intimin, an adhesin in the bacterial cell membrane. Tir and effector protein insertion triggers host cell actin polymerization that leads to an accumulation of actin underneath the attached bacteria, leading to pedestal-like structures that promote the intimate attachment of A/E pathogens to the enterocyte membrane (92,93) **(Figure 3)**. The LEE island, as well as the A/E lesion, are not exclusive of EPEC and EHEC since bacteria such as the mouse pathogen *Citrobacter rodentium*, dog-enteropathogenic *E. coli*, porcine-enteropathogenic *E.coli* and rabbit EPEC (REPEC) also possess the LEE island and share similar characteristics of virulence (79,80,89).

New therapies and strategies have been studied to manage EPEC and EHEC infections. Nevertheless, since these bacteria are poorly pathogenic in mice, there is a limitation for *in vivo* studies in small laboratory models. In this context, *C. rodentium* constitutes an attractive alternative model for understanding the primary mechanisms used by A/E pathogens *in vivo* (94).

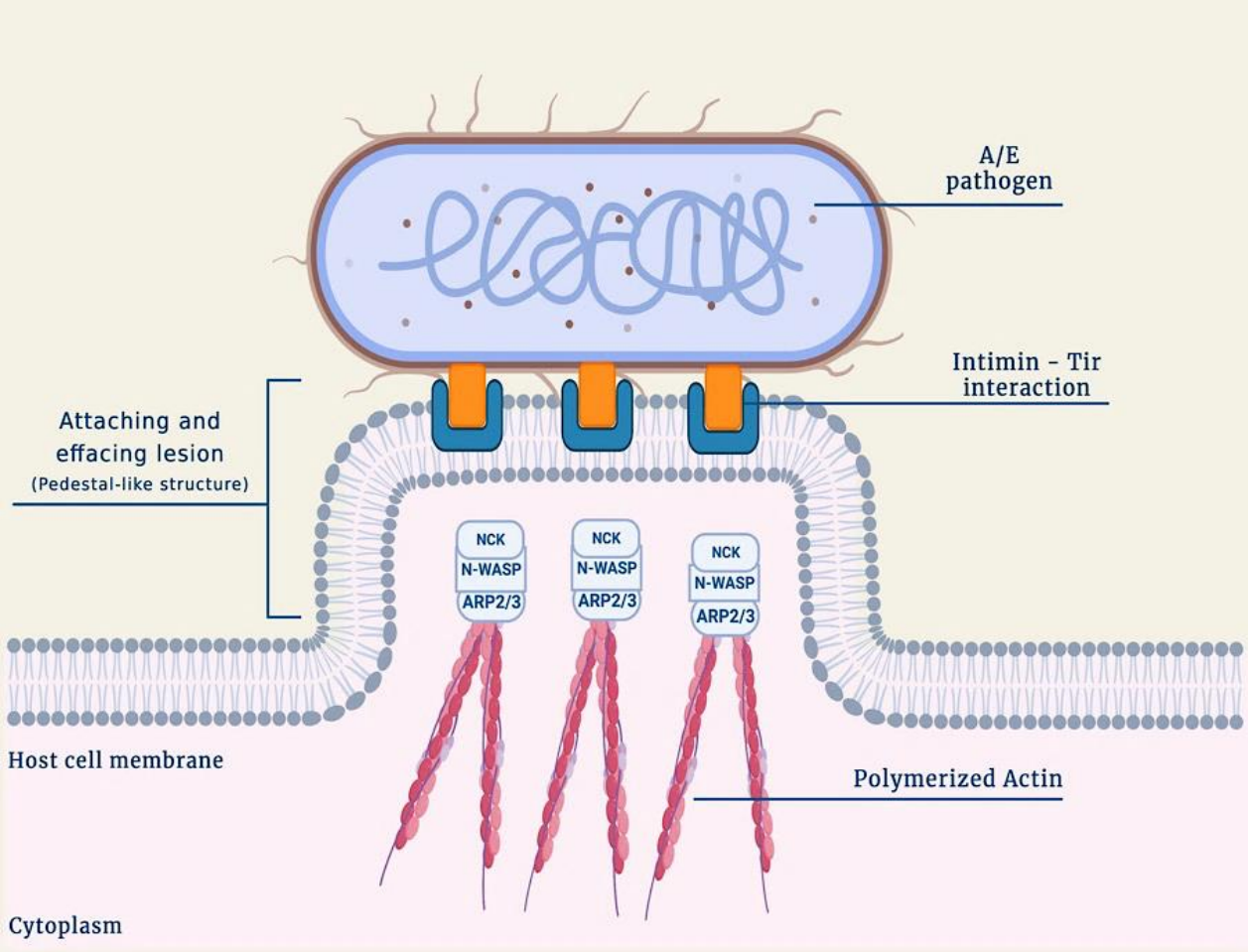

Figure 3. The pedestal-like structures are formed by Attaching and Effacing pathogens. The initial attachment of Attaching and effacing (A/E) pathogens is facilitated by surface adhesins. Subsequently, the receptor (Tir) is translocated within the host cells, binding to the bacterial surface protein intimin in the bacterial cells, promoting close attachment to host cells. The injection of effector proteins and the Tir-Intimin interaction leads to the recruitment of the Nck adaptor to a phosphorylated Tir residue, activating neural Wiskott-Aldrich syndrome protein (N-WASP). This activation triggers a significant pathway for actin polymerization, facilitated by the actin-related protein (Arp) 2/3 complex, culminating in the induction of a pedestal-like structure (Figure created with Biorender).

1.5. *Citrobacter rodentium*: an *in vivo* mouse-pathogen model

C. rodentium is a Gram-negative murine pathogen transmitted via the fecal-oral route that can cause colitis in laboratory mice. It was first described in the literature as atypical *Citrobacter freundii* after an outbreak in mouse colonies between the 1960s and 1970s (95). Later, it was found that *C. rodentium* was a mouse-restricted pathogen with 67% genetic similarity to EHEC and EPEC. The genome of *C. rodentium* also includes the LEE island, thus conferring the ability to form A/E lesions (89,96). Since *C. rodentium* is a natural mouse pathogen that is able to establish infection without antibiotic pretreatment, it has been the principal rodent model for studying diseases caused by A/E pathogens (97).

The susceptibility of inbred mice to *C. rodentium* depends on the genetic background as well as differences in the composition of the gut microbiota. Mouse strains such as C57BL/6 and Swiss Webster develop mild self-limiting disease with a typical infectious cascade, which can be divided into four phases: 1. The establishment phase, 2. the expansion phase, 3. the steady-state phase, and 4, the clearance phase (97). During phase 1, following inoculation and between 1 - 3 days post-infection (DPI), *C. rodentium* initially colonizes the lymphoid patches and adapts to the gut environment (98). In phase 2 (4-8 DPI), *C. rodentium* colonizes the intestinal epithelium cells by loose attachment mediated by fimbriae (94). Next, activation of LEE island genes results in intimate adhesion to the intestinal epithelial cells and initiates the formation of A/E lesions, a process that is mediated by environmental signals (91). The virulence of *C. rodentium* depends on the ability to perform A/E lesions, and studies have shown that the absence of Tir, intimin, and functional T3SS (all encoded by LEE island genes) renders *C. rodentium* to become avirulent (89,96,99).

During phase 3 (8-12 DPI), *C. rodentium* primarily occupies the distal part of the colon and cecum and shedding reaches a plateau at 10^8 and 10^9 colony-forming units (CFU) per gram of feces. Finally, in phase 4 (after 12 DPI), the host starts to clean the infection (97). In more susceptible mouse strains such as C3H and FVB, instead of recovery, the mice rapidly deteriorate after day 8 of infection and ultimately succumb to dehydration (97).

The microbiota is a crucial factor that dictates the host's susceptibility to infections. In *C. rodentium* infection, the microbiota affects the colonization efficiency and influences the outcome of the disease (100). Nevertheless, *C. rodentium* evolved mechanisms to overcome the colonization resistance promoted by the gut microbiota. *C. rodentium* is a facultative anaerobic bacterium capable of surviving in aerobic and anaerobic conditions. The oxygen gradient of the gut is not homogeneous. It varies depending on the spatial location, with higher concentrations at the submucosa and epithelium to near anoxia in the lumen (101). The anoxic environment is beneficial to obligate anaerobic bacteria such as *Clostridia*, which have an essential role in producing short-chain fatty acids such as butyrate (102). Butyrate, for instance, has immunomodulatory activity and is the primary energy source for colonic epithelial cells (103–105). The butyrate metabolism of colonic cells induces mitochondrial β-oxygenation through oxygen consumption, thereby limiting its diffusion and providing a low-oxygen environment at the luminal border (97,103,106). *C. rodentium* can disrupt epithelial mitochondria, forcing IECs to switch their regular metabolism to aerobic glycolysis to produce ATP. This results in lower oxygen consumption by IECs and greater oxygen diffusion into the lumen, thereby providing a fitness advantage for *C. rodentium* (97,106,107).

In mice with both severe and mild disease, *C. rodentium* infection leads to transmissible colonic crypt hyperplasia (TCCH), the hallmark of *C. rodentium* infection (94,95,97,108,109). TCCH is characterized by the induction of tissue damage repair responses due to the excessive proliferation of LGR5+ stem cells and partially differentiated transit-amplifying cells. This process leads to an accumulation of undifferentiated colonocytes at the luminal surface of the colon and loss of mature epithelial cells, such as enterocytes and goblet cells, with the result of a thickened colon mucosa (94,95,97,109). Also, it could facilitate epithelial oxygenation, which is advantageous for *C. rodentium* (110).

1.6. *C. rodentium* and the immune response

During infection, components of the T3SS, Lipopolysaccharide (LPS), an outer membrane portion constitute with a oligosaccharide core, Lipid A and O-antigen (111) or PAMPs of *C. rodentium* are recognized by immune cells, leading to the activation of the downstream adaptor molecule myeloid differentiation primary-response 88 (MYD88) (112). This leads to the activation of NF-kβ, which induces the production of proinflammatory cytokines, including IL-6, IL-12, IL-23, and tumor necrosis factor in myeloid cells (113). Pro-inflammatory responses induce various antimicrobial processes, including the recruitment of neutrophils, macrophages, dendritic cells, and the expression of inducible nitric oxide synthase (INOS) that is essential for controlling *C. rodentium* infection (94). However, the proinflammatory environment also amplifies the proliferation of epithelial cells during TCCH.

The chemokines induced in the colon upon infection promote the influx of neutrophils to the mucosa. In this context, CXCR2 receptor-mediated recruitment of neutrophils is essential for host defense and protection against *C.* rodentium (114). Besides, neutrophil antimicrobial responses such as NET formation ensure *C. rodentium* clearance and mucosal protection during infection (75).

The pro-inflammatory environment initiated by the innate immune system induces the differentiation and expansion of effector T cells such as Th1, Th17, and Th22 (115–117). The production of IL-17 induces expression of the cytokine IL-22 and further promotes the recruitment of neutrophils. Furthermore, ILC3s are an important source of NOD-dependent IL-22 induction (94). Studies have shown that IL-22 is essential for the resolution of *C. rodentium*-mediated disease since the absence of IL-22 leads to higher levels of epithelial damage, systemic bacterial burden, and mortality in mice (39,118). However, the underlying mechanisms of IL-22-mediated protection in the context of A/E pathogens infection remain unknown.

1.7. Chemical signaling in pathogenic *Enterobacteriaceae*

The successful establishment of infection depends on the ability of pathogens to sense the local environment and regulate virulence genes according to changes at the colonized niche. Bacteria can detect environmental fluctuations and establish cell-cell communication through the production and diffusion of small chemical molecules or signals recognized by receptors in their membrane (119). Changes such as increasing bacterial colony numbers, hormones produced by the host, and variations in the sugar concentration can be sensed by bacteria to modulate their response accordingly (79,120–122).

The inner membrane two-component system sensor QseC is a histidine kinase receptor that, together with its cognate regulator QseB, promotes gene modulation in Gram-negative bacteria. QseC senses mammalian adrenergic hormones, norepinephrine, epinephrine, and the autoinducer-3 secreted by bacteria (123,124). The binding of these molecules to the QseC receptor leads to the phosphorylation of QseB, which triggers a chemical signaling cascade that modulates gene expression in bacteria, specifically the upregulation of virulence genes in pathogens (125,126). Pathogens can have access to norepinephrine, and epinephrine is mainly synthesized at the end of postganglionic sympathetic nerve fibers and by chromaffin cells of the adrenal medulla reaching the gut via the bloodstream (17), which could be influenced by the microbial community inhabiting the gut and dietary sources (17,127,128). Besides, studies have shown that immune cells could also be another source of catecholamines (129).

Previous studies have shown that the QseC receptor is not essential for bacterial growth but is involved in pathogenic mechanisms. In *Salmonella enterica serovar* Typhimurium, blockage of the QseC sensor decreases *in vivo* colonization (124). In A/E pathogens, it has been demonstrated that QseC, directly and indirectly, regulates LEE gene expression (120,124,126,130). In EHEC, the blockage of the QseC sensor led to a decrease in the number of epithelial A/E lesions (124). *C. rodentium*-infected C3J mice, which usually succumb to infection, show significantly improved survival rates upon infection with a *qseC* mutant, suggesting that QseC impacts the virulence of *C. rodentium* (131).

Although it is well established that the QseC sensor aids in A/E pathogen colonization, little is known about the role of QseC signaling in these pathogens to evade and resist immune responses during disease establishment. Thus, the contribution of

adrenergic hormone detection via QseC in A/E pathogens during *in vivo* infections, particularly *C. rodentium*, needs further investigation.

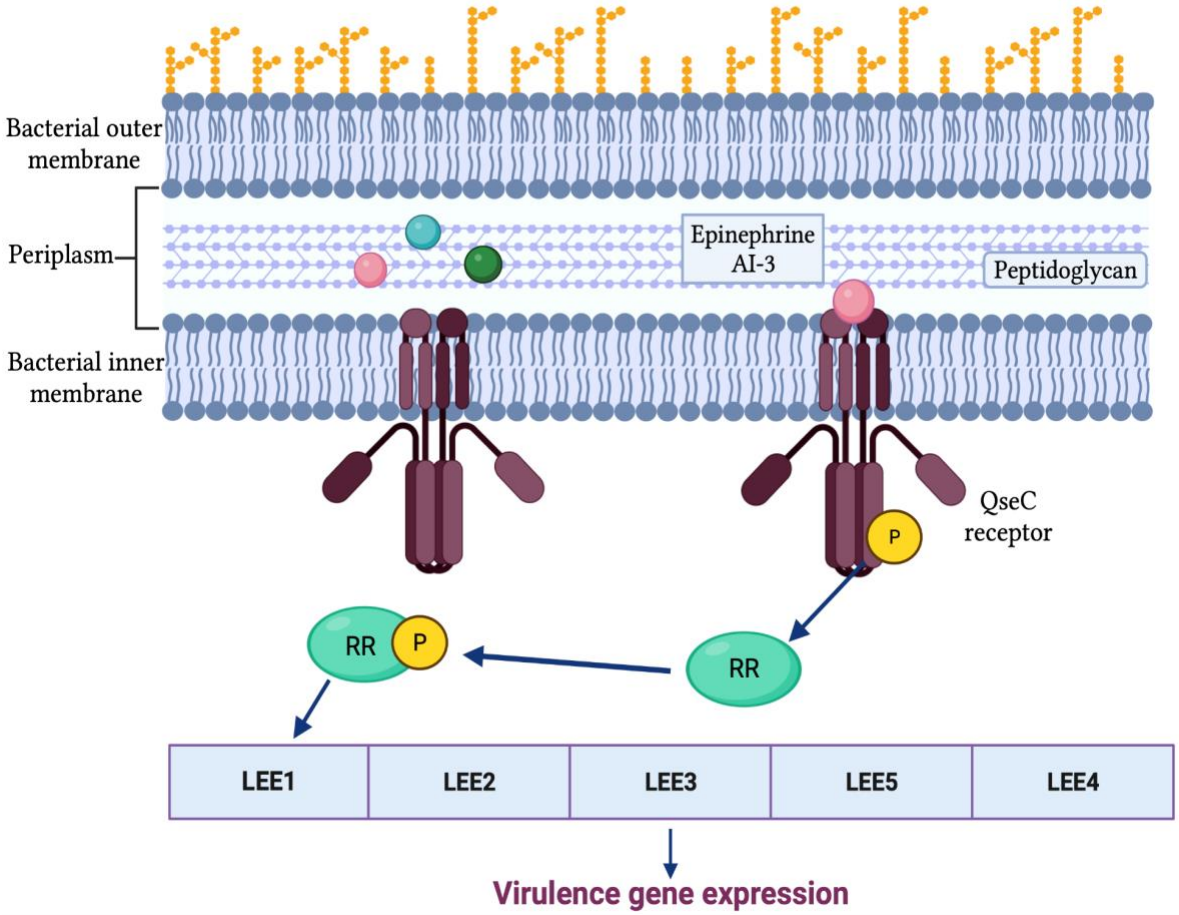

Figure 4. The QseC sensor regulation in A/E pathogens. The histidine kinase sensor QseC detects mammalian adrenergic hormones such as epinephrine and norepinephrine and the bacterial autoinducer-3 produced by bacteria, leading to the phosphorylation of Response Regulators (RR), such as QseB. The phosphorylated RR initiates a signaling cascade that leads to the activation of genes of the Locus of Enterocyte Effacement (LEE) pathogenicity island, thus modulating virulence gene expression in A/E pathogens (Figure created with Biorender).

Chapter IV: Conclusion

Our data demonstrated that through the QseC-mediated signaling, *C. rodentium* has an increased ability to survive neutrophil-mediated immune responses, thereby facilitating systemic spreading *in vivo*. Besides, the QseC sensor is involved in modulating *C. rodentium's* O-antigen chain of LPS, which could be involved in its resistance to neutrophil killing. In addition, mucosal immune mechanisms during *C. rodentium* infections are complex; in our study, CXCR2-mediated neutrophil responses are essential to protect the host from *C. rodentium* colonization, inflammation, and systemic spreading. Moreover, QseC is directly involved in triggering *C. rodentium*-mediated intestinal inflammation and inducing transmissible colonic crypt hyperplasia, the hallmark pathological feature of *C. rodentium* infection. Overall, our *in vivo* and *in vitro* data confirms that the QseC sensor is essential for *C. rodentium* virulence, and it is involved in *C. rodentium's* ability to efficiently evade immune response and establish the disease.

Besides, IL-22 is critical for the resolution of *C. rodentium* infection, which does not seem to be solely dependent on its downstream antimicrobial peptides calprotectin, REG3, Lipocalin-2, and C3, but could also be attributed to IL-22 epithelial mucosal repair mechanisms. These findings broaden our understanding of the role of IL-22 in protecting the host during *C. rodentium* infection.

In summary, our study demonstrated that *C. rodentium* uses the QseC sensor to establish the infection and evade the host response, which involves IL-22-mediated protection for the host to efficiently recover from the infection and clean the disease.

REFERENCES

1. Greenwood-Van Meerveld B, Johnson AC, Grundy D. Gastrointestinal Physiology and Function. In: Greenwood-Van Meerveld B, editor. Gastrointestinal Pharmacology [Internet]. Cham: Springer International Publishing; 2017 [cited 2023 Sep 19]. p. 1–16. (Handbook of Experimental Pharmacology). Available from: https://doi.org/10.1007/164_2016_118

2. Fedoruk MJ, Guidotti TL. Gastrointestinal System. In: Wexler P, editor. Encyclopedia of Toxicology (Second Edition) [Internet]. New York: Elsevier; 2005 [cited 2022 Sep 20]. p. 410–6. Available from: https://www.sciencedirect.com/science/article/pii/B0123694000004464

3. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017 Jun 1;474(11):1823–36.

4. Hou K, Wu ZX, Chen XY, Wang JQ, Zhang D, Xiao C, et al. Microbiota in health and diseases. Signal Transduct Target Ther. 2022 Apr 23;7(1):1–28.

5. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. Nat Rev Microbiol. 2021 Jan;19(1):55–71.

6. Belkaid Y, Hand T. Role of the Microbiota in Immunity and inflammation. Cell. 2014 Mar 27;157(1):121–41.

7. Gieryńska M, Szulc-Dąbrowska L, Struzik J, Mielcarska MB, Gregorczyk-Zboroch KP. Integrity of the Intestinal Barrier: The Involvement of Epithelial Cells and Microbiota—A Mutual Relationship. Anim Open Access J MDPI. 2022 Jan 8;12(2):145.

8. Belkaid Y, Naik S. Compartmentalized and systemic control of tissue immunity by commensals. Nat Immunol. 2013 Jul;14(7):646–53.

9. Perez-Lopez A, Behnsen J, Nuccio SP, Raffatellu M. Mucosal immunity to pathogenic intestinal bacteria. Nat Rev Immunol. 2016 Mar;16(3):135–48.

10. Cornick S, Tawiah A, Chadee K. Roles and regulation of the mucus barrier in the gut. Tissue Barriers. 2015 Jan 15;3(1–2):e982426.

11. Kim JM. Antimicrobial Proteins in Intestine and Inflammatory Bowel Diseases. Intest Res. 2014 Jan;12(1):20–33.

12. Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol. 2010 Mar;10(3):159–69.

13. Soderholm AT, Pedicord VA. Intestinal epithelial cells: at the interface of the microbiota and mucosal immunity. Immunology. 2019 Dec;158(4):267–80.

14. Turner JR. Intestinal mucosal barrier function in health and disease. Nat Rev Immunol. 2009 Nov;9(11):799–809.

15. Murch S. 5 - Gastrointestinal Mucosal Immunology and Mechanisms of Inflammation. In: Wyllie R, Hyams JS, Kay M, editors. Pediatric Gastrointestinal and Liver Disease (Sixth Edition) [Internet]. Philadelphia: Elsevier; 2021 [cited 2022 Oct 15]. p. 40-52.e3. Available from: https://www.sciencedirect.com/science/article/pii/B9780323672931000050

16. McGhee JR, Fujihashi K. Inside the Mucosal Immune System. PLOS Biol. 2012 Sep 25;10(9):e1001397.

17. Mittal R, Debs LH, Patel AP, Nguyen D, Patel K, O'Connor G, et al. Neurotransmitters: The critical modulators regulating gut-brain axis. J Cell Physiol. 2017 Sep;232(9):2359–72.

18. Neutra MR, Mantis NJ, Kraehenbuhl JP. Collaboration of epithelial cells with organized mucosal lymphoid tissues. Nat Immunol. 2001 Nov;2(11):1004–9.

19. Hugenholtz F, de Vos WM. Mouse models for human intestinal microbiota research: a critical evaluation. Cell Mol Life Sci. 2018 Jan 1;75(1):149–60.

20. Vivier E, Malissen B. Innate and adaptive immunity: specificities and signaling hierarchies revisited. Nat Immunol. 2005 Jan;6(1):17–21.

21. Mörbe UM, Jørgensen PB, Fenton TM, von Burg N, Riis LB, Spencer J, et al. Human gut-associated lymphoid tissues (GALT); diversity, structure, and function. Mucosal Immunol. 2021 Jul;14(4):793–802.

22. Akira S, Uematsu S, Takeuchi O. Pathogen Recognition and Innate Immunity. Cell. 2006 Feb 24;124(4):783–801.

23. Ye W, Murata Y. Microbe Associated Molecular Pattern Signaling in Guard Cells. Front Plant Sci [Internet]. 2016 [cited 2023 Oct 3];7. Available from: https://www.frontiersin.org/articles/10.3389/fpls.2016.00583

24. Boller T, Felix G. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. Annu Rev Plant Biol. 2009;60:379–406.

25. Yuan Q, Walker WA. Innate Immunity of the Gut: Mucosal Defense in Health and Disease. J Pediatr Gastroenterol Nutr. 2004 May;38(5):463–73.

26. Luster AD, Alon R, von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. Nat Immunol. 2005 Dec;6(12):1182–90.

27. Martín-Fontecha A, Lanzavecchia A, Sallusto F. Dendritic Cell Migration to Peripheral Lymph Nodes. In: Lombardi G, Riffo-Vasquez Y, editors. Dendritic Cells [Internet]. Berlin, Heidelberg: Springer; 2009 [cited 2022 Oct 18]. p. 31–49. (Handbook of Experimental Pharmacology). Available from: https://doi.org/10.1007/978-3-540-71029-5_2

28. Krummel MF, Bartumeus F, Gérard A. T cell migration, search strategies and mechanisms. Nat Rev Immunol. 2016 Mar;16(3):193–201.

29. Loo TT, Gao Y, Lazarevic V. Transcriptional regulation of CD4+ TH cells that mediate tissue inflammation. J Leukoc Biol. 2018 Dec;104(6):1069–85.

30. Luckheeram RV, Zhou R, Verma AD, Xia B. $CD4^{+}$T cells: differentiation and functions. Clin Dev Immunol. 2012;2012:925135.

31. Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J, et al. Interleukin-22, a TH17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. Nature. 2007 Feb;445(7128):648–51.

32. Valle-Noguera A, Ochoa-Ramos A, Gomez-Sánchez MJ, Cruz-Adalia A. Type 3 Innate Lymphoid Cells as Regulators of the Host-Pathogen Interaction. Front Immunol. 2021 Sep 29;12:748851.

33. Cella M, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JKM, et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. Nature. 2009 Feb 5;457(7230):722–5.

34. Bando JK, Colonna M. Innate lymphoid cell function in the context of adaptive immunity. Nat Immunol. 2016 Jun 21;17(7):783–9.

35. Trifari S, Kaplan CD, Tran EH, Crellin NK, Spits H. Identification of a human helper T cell population that has abundant production of interleukin 22 and is distinct from T(H)-17, T(H)1 and T(H)2 cells. Nat Immunol. 2009 Aug;10(8):864–71.

36. Kreymborg K, Etzensperger R, Dumoutier L, Haak S, Rebollo A, Buch T, et al. IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. J Immunol Baltim Md 1950. 2007 Dec 15;179(12):8098–104.

37. Zhang K, Chen L, Zhu C, Zhang M, Liang C. Current Knowledge of Th22 Cell and IL-22 Functions in Infectious Diseases. Pathogens. 2023 Feb;12(2):176.

38. Keir ME, Yi T, Lu TT, Ghilardi N. The role of IL-22 in intestinal health and disease. J Exp Med. 2020 Feb 13;217(3):e20192195.

39. Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med. 2008 Mar;14(3):282–9.

40. Liang SC, Tan XY, Luxenberg DP, Karim R, Dunussi-Joannopoulos K, Collins M,

et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. J Exp Med. 2006 Oct 2;203(10):2271–9.

41. Raffatellu M, George MD, Akiyama Y, Hornsby MJ, Nuccio SP, Paixao TA, et al. Lipocalin-2 Resistance Confers an Advantage to *Salmonella enterica* Serotype Typhimurium for Growth and Survival in the Inflamed Intestine. Cell Host Microbe. 2009 May 21;5(5):476–86.

42. Vaishnava S, Yamamoto M, Severson KM, Ruhn KA, Yu X, Koren O, et al. The Antibacterial Lectin RegIIIγ Promotes the Spatial Segregation of Microbiota and Host in the Intestine. Science. 2011 Oct 14;334(6053):255–8.

43. Gerner RR, Nuccio SP, Raffatellu M. Iron at the host-microbe interface. Mol Aspects Med. 2020 Oct 1;75:100895.

44. Berger T, Togawa A, Duncan GS, Elia AJ, You-Ten A, Wakeham A, et al. Lipocalin 2-deficient mice exhibit increased sensitivity to *Escherichia coli* infection but not to ischemia-reperfusion injury. Proc Natl Acad Sci U S A. 2006 Feb 7;103(6):1834–9.

45. Corbin BD, Seeley EH, Raab A, Feldmann J, Miller MR, Torres VJ, et al. Metal chelation and inhibition of bacterial growth in tissue abscesses. Science. 2008 Feb 15;319(5865):962–5.

46. Mukherjee S, Bassler BL. Bacterial quorum sensing in complex and dynamically changing environments. Nat Rev Microbiol. 2019 Jun;17(6):371–82.

47. Miki T, Holst O, Hardt WD. The Bactericidal Activity of the C-type Lectin RegIIIβ against Gram-negative Bacteria involves Binding to Lipid A. J Biol Chem. 2012 Oct 5;287(41):34844–55.

48. Castro-Sánchez P, Martín-Villa JM. Gut immune system and oral tolerance. Br J Nutr. 2013 Jan;109 Suppl 2:S3-11.

49. Wu HJ, Wu E. The role of gut microbiota in immune homeostasis and autoimmunity. Gut Microbes. 2012 Jan 1;3(1):4–14.

50. Li MO, Wan YY, Flavell RA. T cell-produced transforming growth factor-beta1 controls T cell tolerance and regulates Th1- and Th17-cell differentiation. Immunity. 2007 May;26(5):579–91.

51. Traxinger BR, Richert-Spuhler LE, Lund JM. Mucosal tissue regulatory T cells are integral in balancing immunity and tolerance at portals of antigen entry. Mucosal Immunol. 2022 May;15(3):398–407.

52. Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, et al. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity. 2008 Apr;28(4):546–58.

53. Bopp T, Becker C, Klein M, Klein-Hessling S, Palmetshofer A, Serfling E, et al. Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. J Exp Med. 2007 Jun 11;204(6):1303–10.

54. Borsellino G, Kleinewietfeld M, Di Mitri D, Sternjak A, Diamantini A, Giometto R, et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression. Blood. 2007 Aug 15;110(4):1225–32.

55. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. J Exp Med. 2007 Aug 6;204(8):1775–85.

56. Chistiakov DA, Bobryshev YV, Kozarov E, Sobenin IA, Orekhov AN. Intestinal mucosal tolerance and impact of gut microbiota to mucosal tolerance. Front Microbiol. 2015 Jan 13;5:781.

57. Johansson-Lindbom B, Svensson M, Pabst O, Palmqvist C, Marquez G, Förster R, et al. Functional specialization of gut CD103+ dendritic cells in the regulation of tissue-selective T cell homing. J Exp Med. 2005 Oct 17;202(8):1063–73.

58. Takiishi T, Fenero CIM, Câmara NOS. Intestinal barrier and gut microbiota: Shaping our immune responses throughout life. Tissue Barriers. 2017 Oct 2;5(4):e1373208.

59. Bohnhoff M, Miller CP. Enhanced susceptibility to *Salmonella* infection in streptomycin-treated mice. J Infect Dis. 1962;111:117–27.

60. Collins FM, Carter PB. Growth of salmonellae in orally infected germfree mice. Infect Immun. 1978 Jul;21(1):41–7.

61. Miller CP, Bohnhoff M, Rifkind D. The Effect of an Antibiotic on the Susceptibility of the Mouse's Intestinal Tract to Salmonella Infection. Trans Am Clin Climatol Assoc. 1957;68:51–8.

62. Sekirov I, Tam NM, Jogova M, Robertson ML, Li Y, Lupp C, et al. Antibiotic-induced perturbations of the intestinal microbiota alter host susceptibility to enteric infection. Infect Immun. 2008 Oct;76(10):4726–36.

63. Baldwin GC. The biology of granulocyte-macrophage colony-stimulating factor: effects on hematopoietic and nonhematopoietic cells. Dev Biol. 1992 Jun;151(2):352–67.

64. Suratt BT, Petty JM, Young SK, Malcolm KC, Lieber JG, Nick JA, et al. Role of the CXCR4/SDF-1 chemokine axis in circulating neutrophil homeostasis. Blood. 2004 Jul 15;104(2):565–71.

65. Sadik CD, Kim ND, Luster AD. Neutrophils cascading their way to inflammation. Trends Immunol. 2011 Oct;32(10):452–60.

66. Furze RC, Rankin SM. Neutrophil mobilization and clearance in the bone marrow. Immunology. 2008 Nov;125(3):281–8.

67. Rosales C. Neutrophil: A Cell with Many Roles in Inflammation or Several Cell Types? Front Physiol. 2018 Feb 20;9:113.

68. Eash KJ, Greenbaum AM, Gopalan PK, Link DC. CXCR2 and CXCR4 antagonistically regulate neutrophil trafficking from murine bone marrow. J Clin Invest. 2010 Jul;120(7):2423–31.

69. Grespan R, Fukada SY, Lemos HP, Vieira SM, Napimoga MH, Teixeira MM, et al. CXCR2-specific chemokines mediate leukotriene B4-dependent recruitment of

neutrophils to inflamed joints in mice with antigen-induced arthritis. Arthritis Rheum. 2008 Jul;58(7):2030–40.

70. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nat Rev Immunol. 2013 Mar;13(3):159–75.

71. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol. 2018 Feb;18(2):134–47.

72. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004 Mar 5;303(5663):1532–5.

73. Urban CF, Reichard U, Brinkmann V, Zychlinsky A. Neutrophil extracellular traps capture and kill Candida albicans yeast and hyphal forms. Cell Microbiol. 2006 Apr;8(4):668–76.

74. Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. J Exp Med. 2010 Aug 30;207(9):1853–62.

75. Saha P, Yeoh BS, Xiao X, Golonka RM, Singh V, Wang Y, et al. PAD4-dependent NETs generation are indispensable for intestinal clearance of *Citrobacter rodentium*. Mucosal Immunol. 2019 May;12(3):761–71.

76. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol. 2016 Aug;14(8):e1002533.

77. Tenaillon O, Skurnik D, Picard B, Denamur E. The population genetics of commensal *Escherichia coli*. Nat Rev Microbiol. 2010 Mar;8(3):207–17.

78. Jang J, Hur HG, Sadowsky M j., Byappanahalli M n., Yan T, Ishii S. Environmental *Escherichia coli*: ecology and public health implications—a review. J Appl Microbiol. 2017;123(3):570–81.

79. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli.* Nat Rev Microbiol. 2004 Feb;2(2):123–40.

80. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic *Escherichia coli*. Clin Microbiol Rev. 2013 Oct;26(4):822–80.

81. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev. 1998 Jan;11(1):142–201.

82. Pakbin B, Brück WM, Rossen JWA. Virulence Factors of Enteric Pathogenic *Escherichia coli*: A Review. Int J Mol Sci. 2021 Sep 14;22(18):9922.

83. Fakhouri F, Zuber J, Frémeaux-Bacchi V, Loirat C. Haemolytic uraemic syndrome. Lancet Lond Engl. 2017 Aug 12;390(10095):681–96.

84. Gomes TAT, Elias WP, Scaletsky ICA, Guth BEC, Rodrigues JF, Piazza RMF, et al. Diarrheagenic *Escherichia coli*. Braz J Microbiol Publ Braz Soc Microbiol. 2016 Dec;47 Suppl 1:3–30.

85. Kimmitt PT, Harwood CR, Barer MR. Toxin gene expression by shiga toxin-producing *Escherichia coli*: the role of antibiotics and the bacterial SOS response. Emerg Infect Dis. 2000;6(5):458–65.

86. Mühlen S, Dersch P. Treatment Strategies for Infections With Shiga Toxin-Producing *Escherichia coli*. Front Cell Infect Microbiol. 2020;10:169.

87. Ochoa TJ, Barletta F, Contreras C, Mercado E. New insights into the epidemiology of enteropathogenic *Escherichia coli* infection. Trans R Soc Trop Med Hyg. 2008 Sep;102(9):852–6.

88. McDaniel TK, Jarvis KG, Donnenberg MS, Kaper JB. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proc Natl Acad Sci U S A. 1995 Feb 28;92(5):1664–8.

89. Deng W, Li Y, Vallance BA, Finlay BB. Locus of enterocyte effacement from *Citrobacter rodentium*: sequence analysis and evidence for horizontal transfer among attaching and effacing pathogens. Infect Immun. 2001 Oct;69(10):6323–35.

90. Franzin FM, Sircili MP. Locus of Enterocyte Effacement: A Pathogenicity Island Involved in the Virulence of Enteropathogenic and Enterohemorragic *Escherichia coli* Subjected to a Complex Network of Gene Regulation. BioMed Res Int. 2015;2015:534738.

91. Deng W, Vallance BA, Li Y, Puente JL, Finlay BB. *Citrobacter rodentium* translocated intimin receptor (Tir) is an essential virulence factor needed for actin condensation, intestinal colonization and colonic hyperplasia in mice. Mol Microbiol. 2003 Apr;48(1):95–115.

92. Gruenheid S, DeVinney R, Bladt F, Goosney D, Gelkop S, Gish GD, et al. Enteropathogenic *E. coli* Tir binds Nck to initiate actin pedestal formation in host cells. Nat Cell Biol. 2001 Sep;3(9):856–9.

93. Kenny B, DeVinney R, Stein M, Reinscheid DJ, Frey EA, Finlay BB. Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. Cell. 1997 Nov 14;91(4):511–20.

94. Collins JW, Keeney KM, Crepin VF, Rathinam VAK, Fitzgerald KA, Finlay BB, et al. *Citrobacter rodentium*: infection, inflammation and the microbiota. Nat Rev Microbiol. 2014 Sep;12(9):612–23.

95. Luperchio SA, Schauer DB. Molecular pathogenesis of *Citrobacter rodentium* and transmissible murine colonic hyperplasia. Microbes Infect. 2001 Apr;3(4):333–40.

96. Petty NK, Bulgin R, Crepin VF, Cerdeño-Tárraga AM, Schroeder GN, Quail MA, et al. The *Citrobacter rodentium* Genome Sequence Reveals Convergent Evolution with Human Pathogenic Escherichia coli. J Bacteriol. 2010 Jan 15;192(2):525–38.

97. Mullineaux-Sanders C, Sanchez-Garrido J, Hopkins EGD, Shenoy AR, Barry R, Frankel G. *Citrobacter rodentium*–host–microbiota interactions: immunity, bioenergetics and metabolism. Nat Rev Microbiol. 2019 Nov;17(11):701–15.

98. Wiles S, Clare S, Harker J, Huett A, Young D, Dougan G, et al. Organ specificity, colonization and clearance dynamics in vivo following oral challenges with the murine

pathogen *Citrobacter rodentium*. Cell Microbiol. 2004 Oct;6(10):963–72.

99. Schauer DB, Falkow S. The eae gene of *Citrobacter freundii* biotype 4280 is necessary for colonization in transmissible murine colonic hyperplasia. Infect Immun. 1993 Nov;61(11):4654–61.

100. Willing BP, Vacharaksa A, Croxen M, Thanachayanont T, Finlay BB. Altering Host Resistance to Infections through Microbial Transplantation. PLOS ONE. 2011 Oct 28;6(10):e26988.

101. Espey MG. Role of oxygen gradients in shaping redox relationships between the human intestine and its microbiota. Free Radic Biol Med. 2013 Feb 1;55:130–40.

102. Guo P, Zhang K, Ma X, He P. *Clostridium* species as probiotics: potentials and challenges. J Anim Sci Biotechnol. 2020 Feb 20;11(1):24.

103. Litvak Y, Byndloss MX, Bäumler AJ. Colonocyte metabolism shapes the gut microbiota. Science. 2018 Nov 30;362(6418):eaat9076.

104. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell Metab. 2011 May 4;13(5):517–26.

105. Corrêa-Oliveira R, Fachi JL, Vieira A, Sato FT, Vinolo MAR. Regulation of immune cell function by short-chain fatty acids. Clin Transl Immunol. 2016 Apr 22;5(4):e73.

106. Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, et al. Crosstalk between Microbiota-Derived Short-Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function. Cell Host Microbe. 2015 May 13;17(5):662–71.

107. Berger CN, Crepin VF, Roumeliotis TI, Wright JC, Carson D, Pevsner-Fischer M, et al. *Citrobacter rodentium* Subverts ATP Flux and Cholesterol Homeostasis in Intestinal Epithelial Cells In Vivo. Cell Metab. 2017 Nov 7;26(5):738-752.e6.

108. Barthold SW, Coleman GL, Bhatt PN, Osbaldiston GW, Jonas AM. The etiology of transmissible murine colonic hyperplasia. Lab Anim Sci. 1976 Dec;26(6 Pt 1):889–94.

109. Barthold SW, Coleman GL, Jacoby RO, Livestone EM, Jonas AM. Transmissible murine colonic hyperplasia. Vet Pathol. 1978 Mar;15(2):223–36.

110. Lopez CA, Miller BM, Rivera-Chávez F, Velazquez EM, Byndloss MX, Chávez-Arroyo A, et al. Virulence factors enhance Citrobacter rodentium expansion through aerobic respiration. Science. 2016 Sep 16;353(6305):1249–53.

111. Whitfield C, Trent MS. Biosynthesis and export of bacterial lipopolysaccharides. Annu Rev Biochem. 2014;83:99–128.

112. Gibson DL, Ma C, Bergstrom KSB, Huang JT, Man C, Vallance BA. MyD88 signalling plays a critical role in host defence by controlling pathogen burden and promoting epithelial cell homeostasis during *Citrobacter rodentium*-induced colitis. Cell Microbiol. 2008 Mar;10(3):618–31.

113. Zhang JM, An J. Cytokines, Inflammation and Pain. Int Anesthesiol Clin. 2007;45(2):27–37.

114. Spehlmann ME, Dann SM, Hruz P, Hanson E, McCole DF, Eckmann L. CXCR2-Dependent Mucosal Neutrophil Influx Protects against Colitis-Associated Diarrhea Caused by an Attaching/Effacing Lesion-Forming Bacterial Pathogen. J Immunol Baltim Md 1950. 2009 Sep 1;183(5):3332–43.

115. Basu R, O'Quinn DB, Silberger DJ, Schoeb TR, Fouser L, Ouyang W, et al. Th22 cells are an important source of IL-22 for host protection against enteropathogenic bacteria. Immunity. 2012 Dec 14;37(6):1061–75.

116. Geddes K, Rubino SJ, Magalhaes JG, Streutker C, Le Bourhis L, Cho JH, et al. Identification of an innate T helper type 17 response to intestinal bacterial pathogens. Nat Med. 2011 Jun 12;17(7):837–44.

117. Simmons CP, Clare S, Ghaem-Maghami M, Uren TK, Rankin J, Huett A, et al. Central role for B lymphocytes and CD4+ T cells in immunity to infection by the attaching and effacing pathogen *Citrobacter rodentium*. Infect Immun. 2003 Sep;71(9):5077–86.

118. Sakamoto K, Kim YG, Hara H, Kamada N, Caballero-Flores G, Tolosano E, et al. IL-22 Controls Iron-Dependent Nutritional Immunity Against Systemic Bacterial Infections. Sci Immunol. 2017 Feb;2(8):eaai8371.

119. Sperandio V, Torres AG, Jarvis B, Nataro JP, Kaper JB. Bacteria-host communication: the language of hormones. Proc Natl Acad Sci U S A. 2003 Jul 22;100(15):8951–6.

120. Sircili MP, Walters M, Trabulsi LR, Sperandio V. Modulation of Enteropathogenic *Escherichia coli* Virulence by Quorum Sensing. Infect Immun. 2004 Apr;72(4):2329–37.

121. Reading NC, Torres AG, Kendall MM, Hughes DT, Yamamoto K, Sperandio V. A novel two-component signaling system that activates transcription of an enterohemorrhagic *Escherichia coli* effector involved in remodeling of host actin. J Bacteriol. 2007 Mar;189(6):2468–76.

122. Njoroge JW, Nguyen Y, Curtis MM, Moreira CG, Sperandio V. Virulence meets metabolism: Cra and KdpE gene regulation in enterohemorrhagic *Escherichia coli*. mBio. 2012 Oct 16;3(5):e00280-00212.

123. Sperandio V, Torres AG, Kaper JB. Quorum sensing Escherichia coli regulators B and C (QseBC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in *E. coli.* Mol Microbiol. 2002 Feb;43(3):809–21.

124. Rasko DA, Moreira CG, Li DR, Reading NC, Ritchie JM, Waldor MK, et al. Targeting QseC signaling and virulence for antibiotic development. Science. 2008 Aug 22;321(5892):1078–80.

125. Moreira CG, Weinshenker D, Sperandio V. QseC mediates *Salmonella* enterica serovar typhimurium virulence in vitro and in vivo. Infect Immun. 2010 Mar;78(3):914–26.

126. Njoroge J, Sperandio V. Enterohemorrhagic *Escherichia coli* virulence regulation by two bacterial adrenergic kinases, QseC and QseE. Infect Immun. 2012 Feb;80(2):688–703.

127. Asano Y, Hiramoto T, Nishino R, Aiba Y, Kimura T, Yoshihara K, et al. Critical role of gut microbiota in the production of biologically active, free catecholamines in the gut lumen of mice. Am J Physiol Gastrointest Liver Physiol. 2012 Dec 1;303(11):G1288-1295.

128. Strandwitz P. Neurotransmitter modulation by the gut microbiota. Brain Res. 2018 Aug 15;1693(Pt B):128–33.

129. Flierl MA, Rittirsch D, Huber-Lang M, Sarma JV, Ward PA. Catecholamines—Crafty Weapons in the Inflammatory Arsenal of Immune/Inflammatory Cells or Opening Pandora's Box? Mol Med. 2008 Mar;14(3):195–204.

130. Walters M, Sperandio V. Autoinducer 3 and epinephrine signaling in the kinetics of locus of enterocyte effacement gene expression in enterohemorrhagic *Escherichia coli*. Infect Immun. 2006 Oct;74(10):5445–55.

131. Moreira CG, Russell R, Mishra AA, Narayanan S, Ritchie JM, Waldor MK, et al. Bacterial Adrenergic Sensors Regulate Virulence of Enteric Pathogens in the Gut. mBio. 2016 Jun 7;7(3):e00826-16.

132. Hoiseth SK, Stocker BA. Aromatic-dependent *Salmonella* Typhimurium are non-virulent and effective as live vaccines. Nature. 1981 May 21;291(5812):238–9.

133. Moreira CG, Sperandio V. Interplay between the QseC and QseE Bacterial Adrenergic Sensor Kinases in *Salmonella enterica* Serovar Typhimurium Pathogenesis. Infect Immun. 2012 Dec;80(12):4344–53.

134. Perez-Lopez A, Silva S, Lee MH, Dillon N, Brandt SL, Gerner RR, et al. CCL28 modulates neutrophil responses during infection with mucosal pathogens [Internet]. bioRxiv; 2022 [cited 2023 Nov 17]. p. 2021.03.19.436197. Available from: https://www.biorxiv.org/content/10.1101/2021.03.19.436197v2

135. Frey A, Di Canzio J, Zurakowski D. A statistically defined endpoint titer determination method for immunoassays. J Immunol Methods. 1998 Dec 1;221(1–2):35–41.

136. Cao DY, Giani JF, Veiras LC, Bernstein EA, Okwan-Duodu D, Ahmed F, et al. An ACE inhibitor reduces bactericidal activity of human neutrophils in vitro and impairs mouse neutrophil activity in vivo. Sci Transl Med. 2021 Jul 28;13(604):eabj2138.

137. Moschen AR, Gerner RR, Wang J, Klepsch V, Adolph TE, Reider SJ, et al. Lipocalin 2 Protects from Inflammation and Tumorigenesis Associated with Gut Microbiota Alterations. Cell Host Microbe. 2016 Apr 13;19(4):455–69.

138. Weiss G, Schaible UE. Macrophage defense mechanisms against intracellular bacteria. Immunol Rev. 2015;264(1):182–203.

139. Marketon MM, DePaolo RW, DeBord KL, Jabri B, Schneewind O. Plague bacteria target immune cells during infection. Science. 2005 Sep 9;309(5741):1739–41.

140. Vareechon C, Zmina SE, Karmakar M, Pearlman E, Rietsch A. Pseudomonas aeruginosa Effector ExoS Inhibits ROS Production in Human Neutrophils. Cell Host Microbe. 2017 May 10;21(5):611-618.e5.

141. Kehl-Fie TE, Chitayat S, Hood MI, Damo S, Restrepo N, Garcia C, et al. Nutrient Metal Sequestration by Calprotectin Inhibits Bacterial Superoxide Defense, Enhancing Neutrophil Killing of *Staphylococcus aureus*. Cell Host Microbe. 2011 Aug 18;10(2):158–64.

142. Miyasaki KT, Bodeau AL, Murthy AR, Lehrer RI. In vitro antimicrobial activity of the human neutrophil cytosolic S-100 protein complex, calprotectin, against *Capnocytophaga sputigena*. J Dent Res. 1993 Feb;72(2):517–23.

143. Zygiel EM, Nolan EM. Transition Metal Sequestration by the Host-Defense Protein Calprotectin. Annu Rev Biochem. 2018 Jun 20;87:621–43.

144. Halverson TWR, Wilton M, Poon KKH, Petri B, Lewenza S. DNA Is an Antimicrobial Component of Neutrophil Extracellular Traps. PLoS Pathog. 2015 Jan 15;11(1):e1004593.

145. Corbett JA, Tilton RG, Chang K, Hasan KS, Ido Y, Wang JL, et al. Aminoguanidine,

a novel inhibitor of nitric oxide formation, prevents diabetic vascular dysfunction. Diabetes. 1992 Apr;41(4):552–6.

146. Corbett JA, McDaniel ML. The Use of Aminoguanidine, a Selective iNOS Inhibitor, to Evaluate the Role of Nitric Oxide in the Development of Autoimmune Diabetes. Methods San Diego Calif. 1996 Aug;10(1):21–30.

147. Farhad AR, Razavi SM, Nejad PA. The use of aminoguanidine, a selective inducible nitric oxide synthase inhibitor, to evaluate the role of nitric oxide on periapical healing. Dent Res J. 2011;8(4):197–202.

148. Ruest MK, Dennis JJ. The Exploration of Complement-Resistance Mechanisms of Pathogenic Gram-Negative Bacteria to Support the Development of Novel Therapeutics. Pathogens. 2022 Aug 18;11(8):931.

149. West NP, Sansonetti P, Mounier J, Exley RM, Parsot C, Guadagnini S, et al. Optimization of virulence functions through glucosylation of Shigella LPS. Science. 2005 Feb 25;307(5713):1313–7.

150. Murray GL, Attridge SR, Morona R. Altering the length of the lipopolysaccharide O antigen has an impact on the interaction of *Salmonella enterica* serovar Typhimurium with macrophages and complement. J Bacteriol. 2006 Apr;188(7):2735–9.

151. Maldonado RF, Sá-Correia I, Valvano MA. Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS Microbiol Rev. 2016 Jul;40(4):480–93.

152. Berry MC, McGhee GC, Zhao Y, Sundin GW. Effect of a waaL mutation on lipopolysaccharide composition, oxidative stress survival, and virulence in *Erwinia amylovora*. FEMS Microbiol Lett. 2009 Feb;291(1):80–7.

153. Duerr CU, Zenk SF, Chassin C, Pott J, Gütle D, Hensel M, et al. O-antigen delays lipopolysaccharide recognition and impairs antibacterial host defense in murine intestinal epithelial cells. PLoS Pathog. 2009 Sep;5(9):e1000567.

154. Mevorach D. Opsonization of apoptotic cells. Implications for uptake and autoimmunity. Ann N Y Acad Sci. 2000;926:226–35.

155. Charles A Janeway J, Travers P, Walport M, Shlomchik MJ. The complement system and innate immunity. In: Immunobiology: The Immune System in Health and Disease 5th edition [Internet]. Garland Science; 2001 [cited 2023 Oct 15]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK27100/

156. Lebeis SL, Bommarius B, Parkos CA, Sherman MA, Kalman D. TLR Signaling Mediated by MyD88 Is Required for a Protective Innate Immune Response by Neutrophils to *Citrobacter rodentium*. J Immunol. 2007 Jul 1;179(1):566–77.

157. Kamada N, Kim YG, Sham HP, Vallance BA, Puente JL, Martens EC, et al. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. Science. 2012 Jun 8;336(6086):1325–9.

158. Ozuna H, Uriarte SM, Demuth DR. The Hunger Games: *Aggregatibacter actinomycetemcomitans* Exploits Human Neutrophils As an Epinephrine Source for Survival. Front Immunol. 2021;12:707096.

159. De Araujo C, Balestrino D, Roth L, Charbonnel N, Forestier C. Quorum sensing affects biofilm formation through lipopolysaccharide synthesis in *Klebsiella pneumoniae*. Res Microbiol. 2010 Sep;161(7):595–603.

160. Mokrievich AN, Kondakova AN, Valade E, Platonov ME, Vakhrameeva GM, Shaikhutdinova RZ, et al. Biological properties and structure of the lipopolysaccharide of a vaccine strain of *Francisella tularensis* generated by inactivation of a quorum sensing system gene qseC. Biochem Mosc. 2010 Apr 1;75(4):443–51.

161. Wang Y, Huang Y, Chase RC, Li T, Ramai D, Li S, et al. Global Burden of Digestive Diseases: A Systematic Analysis of the Global Burden of Diseases Study, 1990 to 2019. Gastroenterology. 2023 Sep 1;165(3):773-783.e15.

162. Sonnenberg GF, Fouser LA, Artis D. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. Nat Immunol. 2011

May;12(5):383–90.

163. Cash HL, Whitham CV, Behrendt CL, Hooper LV. Symbiotic Bacteria Direct Expression of an Intestinal Bactericidal Lectin. Science. 2006 Aug 25;313(5790):1126–30.

164. Shin JH, Seeley RJ. Reg3 Proteins as Gut Hormones? Endocrinology. 2019 May 9;160(6):1506–14.

165. Kinnebrew MA, Ubeda C, Zenewicz LA, Smith N, Flavell RA, Pamer EG. Bacterial flagellin stimulates TLR5-dependent defense against vancomycin-resistant Enterococcus infection. J Infect Dis. 2010 Feb 15;201(4):534–43.

166. Hasegawa M, Yada S, Zhen Liu M, Kamada N, Muñoz-Planillo R, Do N, et al. Interleukin-22 Regulates the Complement System to Promote Resistance against Pathobionts after Pathogen-Induced Intestinal Damage. Immunity. 2014 Oct 16;41(4):620–32.

167. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, et al. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature. 2004 Dec 16;432(7019):917–21.

168. Feinen B, Russell MW. Contrasting Roles of IL-22 and IL-17 in Murine Genital Tract Infection by Neisseria gonorrhoeae. Front Immunol. 2012;3:11.

169. Graham AC, Carr KD, Sieve AN, Indramohan M, Break TJ, Berg RE. IL-22 Production Is Regulated by IL-23 During Listeria monocytogenes Infection but Is Not Required for Bacterial Clearance or Tissue Protection. PLOS ONE. 2011 Feb 15;6(2):e17171.

170. Behnsen J, Jellbauer S, Wong CP, Edwards RA, George MD, Ouyang W, et al. The cytokine IL-22 promotes pathogen colonization by suppressing related commensal bacteria. Immunity. 2014 Feb 20;40(2):262–73.

171. Grizotte-Lake M, Zhong G, Duncan K, Kirkwood J, Iyer N, Smolenski I, et al.

Commensals Suppress Intestinal Epithelial Cell Retinoic Acid Synthesis to Regulate Interleukin-22 Activity and Prevent Microbial Dysbiosis. Immunity. 2018 Dec 18;49(6):1103-1115.e6.

172. Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, et al. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med. 2008 Mar;14(3):275–81.

173. Vallance BA, Deng W, Jacobson K, Finlay BB. Host Susceptibility to the Attaching and Effacing Bacterial Pathogen *Citrobacter rodentium*. Infect Immun. 2003 Jun;71(6):3443–53.

174. Tsai PY, Zhang B, He WQ, Zha JM, Odenwald MA, Singh G, et al. IL-22 Upregulates Epithelial Claudin-2 to Drive Diarrhea and Enteric Pathogen Clearance. Cell Host Microbe. 2017 Jun 14;21(6):671-681.e4.

175. Pham TAN, Clare S, Goulding D, Arasteh JM, Stares MD, Browne HP, et al. Epithelial IL-22RA1-mediated fucosylation promotes intestinal colonization resistance to an opportunistic pathogen. Cell Host Microbe. 2014 Oct 8;16(4):504–16.

176. Curtis MM, Hu Z, Klimko C, Narayanan S, Deberardinis R, Sperandio V. The gut commensal Bacteroides thetaiotaomicron exacerbates enteric infection through modification of the metabolic landscape. Cell Host Microbe. 2014 Dec 10;16(6):759–69.

177. Manitz MP, Horst B, Seeliger S, Strey A, Skryabin BV, Gunzer M, et al. Loss of S100A9 (MRP14) results in reduced interleukin-8-induced CD11b surface expression, a polarized microfilament system, and diminished responsiveness to chemoattractants in vitro. Mol Cell Biol. 2003 Feb;23(3):1034–43.

178. Wessels MR, Butko P, Ma M, Warren HB, Lage AL, Carroll MC. Studies of group B streptococcal infection in mice deficient in complement component C3 or C4 demonstrate an essential role for complement in both innate and acquired immunity. Proc Natl Acad Sci U S A. 1995 Dec 5;92(25):11490–4.

179. Wang L, Fouts DE, Stärkel P, Hartmann P, Chen P, Llorente C, et al. Intestinal

REG3 Lectins Protect against Alcoholic Steatohepatitis by Reducing Mucosa-Associated Microbiota and Preventing Bacterial Translocation. Cell Host Microbe. 2016 Feb 10;19(2):227–39.

180. Wan Z, Fan Y, Liu X, Xue J, Han Z, Zhu C, et al. NLRP3 inflammasome promotes diabetes-induced endothelial inflammation and atherosclerosis. Diabetes Metab Syndr Obes Targets Ther. 2019 Sep 20;12:1931–42.

181. Overbergh L, Giulietti A, Valckx D, Decallonne B, Bouillon R, Mathieu C. The Use of Real-Time Reverse Transcriptase PCR for the Quantification of Cytokine Gene Expression. J Biomol Tech JBT. 2003 Mar;14(1):33–43.

182. Godinez I, Haneda T, Raffatellu M, George MD, Paixão TA, Rolán HG, et al. T cells help to amplify inflammatory responses induced by *Salmonella enterica* serotype Typhimurium in the intestinal mucosa. Infect Immun. 2008 May;76(5):2008–17.

183. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The Neutrophil Lipocalin NGAL Is a Bacteriostatic Agent that Interferes with Siderophore-Mediated Iron Acquisition. Mol Cell. 2002 Nov 1;10(5):1033–43.

184. Hood MI, Mortensen BL, Moore JL, Zhang Y, Kehl-Fie TE, Sugitani N, et al. Identification of an *Acinetobacter baumannii* Zinc Acquisition System that Facilitates Resistance to Calprotectin-mediated Zinc Sequestration. PLOS Pathog. 2012 Dec 6;8(12):e1003068.

185. Diaz-Ochoa VE, Lam D, Lee CS, Klaus S, Behnsen J, Liu JZ, et al. Salmonella Mitigates Oxidative Stress and Thrives in the Inflamed Gut by Evading Calprotectin-Mediated Manganese Sequestration. Cell Host Microbe. 2016 Jun 8;19(6):814–25.

186. Gaddy JA, Radin JN, Cullen TW, Chazin WJ, Skaar EP, Trent MS, et al. Helicobacter pylori Resists the Antimicrobial Activity of Calprotectin via Lipid A Modification and Associated Biofilm Formation. mBio. 2015 Dec 8;6(6):10.1128/mbio.01349-15.

187. Sanos SL, Vonarbourg C, Mortha A, Diefenbach A. Control of epithelial cell

function by interleukin-22-producing RORγt+ innate lymphoid cells. Immunology. 2011 Apr;132(4):453–65.

188. Brandl K, Plitas G, Schnabl B, DeMatteo RP, Pamer EG. MyD88-mediated signals induce the bactericidal lectin RegIII gamma and protect mice against intestinal Listeria monocytogenes infection. J Exp Med. 2007 Aug 6;204(8):1891–900.

189. Lo BC, Shin SB, Hernaez DC, Refaeli I, Yu HB, Goebeler V, et al. IL-22 Preserves Gut Epithelial Integrity and Promotes Disease Remission during Chronic *Salmonella* Infection. J Immunol. 2019 Feb 1;202(3):956–65.

190. Jones RG, Li X, Gray PD, Kuang J, Clayton F, Samowitz WS, et al. Conditional deletion of β1 integrins in the intestinal epithelium causes a loss of Hedgehog expression, intestinal hyperplasia, and early postnatal lethality. J Cell Biol. 2006 Nov 6;175(3):505–14.

191. Bouvard D, Brakebusch C, Gustafsson E, Aszódi A, Bengtsson T, Berna A, et al. Functional consequences of integrin gene mutations in mice. Circ Res. 2001 Aug 3;89(3):211–23.

192. Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S. Claudin-1 and -2: Novel Integral Membrane Proteins Localizing at Tight Junctions with No Sequence Similarity to Occludin. J Cell Biol. 1998 Jun 29;141(7):1539–50.

193. Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S, et al. Occludin: a novel integral membrane protein localizing at tight junctions. J Cell Biol. 1993 Dec;123(6 Pt 2):1777–88.

194. Schneider MR, Dahlhoff M, Horst D, Hirschi B, Trülzsch K, Müller-Höcker J, et al. A Key Role for E-cadherin in Intestinal Homeostasis and Paneth Cell Maturation. PLoS ONE. 2010 Dec 14;5(12):e14325.

195. Gustafsson JK, Navabi N, Rodriguez-Piñeiro AM, Alomran AHA, Premaratne P, Fernandez HR, et al. Dynamic Changes in Mucus Thickness and Ion Secretion during *Citrobacter rodentium* Infection and Clearance. PLOS ONE. 2013 Dec 30;8(12):e84430.

196. Wlodarska M, Willing B, Keeney KM, Menendez A, Bergstrom KS, Gill N, et al. Antibiotic Treatment Alters the Colonic Mucus Layer and Predisposes the Host to Exacerbated *Citrobacter rodentium*-Induced Colitis. Infect Immun. 2011 Apr;79(4):1536–45.

197. Parks OB, Pociask DA, Hodzic Z, Kolls JK, Good M. Interleukin-22 Signaling in the Regulation of Intestinal Health and Disease. Front Cell Dev Biol. 2015;3:85.

198. Zhu Q, Korenfeld D, Suarez-Fueyo A, Graham S, Jin L, Punit S, et al. Epithelial dysfunction is prevented by IL-22 treatment in a *Citrobacter rodentium*-induced colitis model that shares similarities with inflammatory bowel disease. Mucosal Immunol. 2022 Jun;15(6):1338–49.

199. Begg SL. The role of metal ions in the virulence and viability of bacterial pathogens. Biochem Soc Trans. 2019 Feb 28;47(1):77–87.

200. Sheldon JR, Laakso HA, Heinrichs DE. Iron Acquisition Strategies of Bacterial Pathogens. Microbiol Spectr. 2016 Mar 18;4(2):10.1128/microbiolspec.vmbf-0010–2015.

201. Gerner RR, Hossain S, Sargun A, Siada K, Norton GJ, Zheng T, et al. Siderophore Immunization Restricted Colonization of Adherent-Invasive *Escherichia coli* and Ameliorated Experimental Colitis. mBio. 2022 Sep 12;13(5):e02184-22.

202. Behnsen J, Zhi H, Aron AT, Subramanian V, Santus W, Lee MH, et al. Siderophore-mediated zinc acquisition enhances enterobacterial colonization of the inflamed gut. Nat Commun. 2021 Dec 1;12(1):7016.

203. Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT. Complement System Part II: Role in Immunity. Front Immunol. 2015 May 26;6:257.

204. Belzer C, Liu Q, Carroll MC, Bry L. The role of specific igG and complement in combating a primary mucosal infection of the gut epithelium. Eur J Microbiol Immunol. 2011 Dec;1(4):311–8.

205. Mukherjee S, Zheng H, Derebe MG, Callenberg KM, Partch CL, Rollins D, et al.

Antibacterial membrane attack by a pore-forming intestinal C-type lectin. Nature. 2014 Jan;505(7481):103–7.

206. Choi SM, McAleer JP, Zheng M, Pociask DA, Kaplan MH, Qin S, et al. Innate Stat3-mediated induction of the antimicrobial protein Reg3γ is required for host defense against MRSA pneumonia. J Exp Med. 2013 Mar 11;210(3):551–61.

207. Brandl K, Plitas G, Mihu CN, Ubeda C, Jia T, Fleisher M, et al. Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature. 2008 Oct 9;455(7214):804–7.

208. Zheng Y, Valdez PA, Danilenko DM, Hu Y, Sa SM, Gong Q, et al. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. Nat Med. 2008 Mar;14(3):282–9.

209. Sugimoto K, Ogawa A, Mizoguchi E, Shimomura Y, Andoh A, Bhan AK, et al. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. J Clin Invest. 2008 Feb;118(2):534–44.

210. Bry L, Falk PG, Midtvedt T, Gordon JI. A Model of Host-Microbial Interactions in an Open Mammalian Ecosystem. Science. 1996 Sep 6;273(5280):1380–3.

211. Pickard JM, Maurice CF, Kinnebrew MA, Abt MC, Schenten D, Golovkina T, et al. Rapid fucosylation of intestinal epithelium sustains host-commensal symbiosis in sickness. Nature. 2014 Oct 30;514(7524):638–41.