

Citrobacter rodentium, a Gut Pathogen: The Yin and the Yang of Its Pathophysiology, Immunity and Clinical Manifestation in Mice

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Abstract

Pathogenic strains of *E. coli* including enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC) are principle causes for diarrhoea in many parts of the globe. *Citrobacter rodentium* (*C. rodentium*), a gram negative bacterium, is a murine pathogen that also utilizes type III secretion system and similar virulence factors to EPEC and EHEC and forms comparable attaching/effacing lesions in the intestines as EPEC and EHEC. The infection caused by *C. rodentium* in mice is usually self-limiting and results in only minor systemic effects with higher mortality in some susceptible mouse strains. All these characteristics have made the bacteria a commonly used model to study host immune responses to pathogenic *E. coli* infection. In this review, we focus on the impact of virulence factors of the pathogen; different immune components involved in the immune response and summarize their role during *C. rodentium* infection.

Keywords

Citrobacter rodentium, Attaching and Effacing Pathogen, Locus of Enterocyte Effacement, Transmissible Murine Colonic Hyperplasia, Colitis, Mucosal Immune Response

1. Introduction

Escherichia coli is a frequent commensal organism of human intestine, often

colonizing immediately after birth and usually remaining for decades [1]. However, some *E. coli* strains exhibit pathogenic potential, when they acquire certain virulence associated genes [2]. Pathogenic strains of *E. coli* including enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) are the leading causes of diarrheal outbreak in most parts of the world [3]. EPEC is a major source of diarrhoea in children under two years of age causing deaths of a million each year in developing countries [4] [5]. EHEC causes bloody diarrhoea in children and elderly in developed countries with approximately 73,000 cases reported each year in the United states [6] [7] which can cause fatal diseases like haemorrhagic colitis and haemolytic uremic syndrome [8] [9]. The hallmark of EPEC and EHEC induced pathology is that they populate in the intestinal epithelium through the development of attaching and effacing (A/E) lesions [10]. As these pathogens are a profound global health concern understanding their clinical manifestation, pathogenesis and immunity has become the focus of extensive investigation. However, EPEC and EHEC elicit narrow range of host specificity and do not elicit a pertinent disease in laboratory animal genera, which makes it difficult to study EPEC and EHEC pathogenesis [11]. *Citrobacter rodentium* is an accepted mouse pathogen that uses comparable virulence factors as EPEC and EHEC and forms analogous A/E lesions in the distal colon of mice [12] [13] [14]. Subsequently, *C. rodentium* has become a commonly used animal representative to explore the immune responses to pathogenic *E. coli* contamination in humans.

2. *C. rodentium*, an Attaching and Effacing Pathogen

C. rodentium (formerly known as *Citrobacter freundii* biotype 4280) a non-motile, gram-negative bacteria in the family of Enterobacteriaceae, is recognised as the contributing species of transmissible murine colonic hyperplasia (TMCH) [15]. The disease is spread through the faecal-oral route and clinical symptoms occur predominantly in weanling mice [16]. Overall, the infection is self-curing in adult mice with higher mortality in some susceptible mouse strains [13]. In general, the infected mice exhibit decrease in body weight, excretion of soft faecal pellets with diarrhoea in severe cases and crypt hyperplasia.

Following entry to the body via oral route, *C. rodentium* colonizes the caecal patch, a type of lymphoid tissue in caecum, from where the bacteria gradually progress to colonise distal colon [17] [18] [19]. For colonization, the bacteria triggers localised damage of brush border microvilli, which mediates bacteria to attach to the epithelial cell surface. This attachment subsequently produces actin-rich structures of epithelial cells which resemble pedestal and they form these lesions beneath the associated bacteria [20]. The ultra-structural alterations are known as attaching and effacing lesions [21] [22] [23]. The effector proteins essential for the generation of A/E lesion are transported to an intestinal cell through a type III secretion system (Figure 1), a needle-like structure, which ensures a smooth passage of bacterial proteins directly in to the host cell cytoplasm.

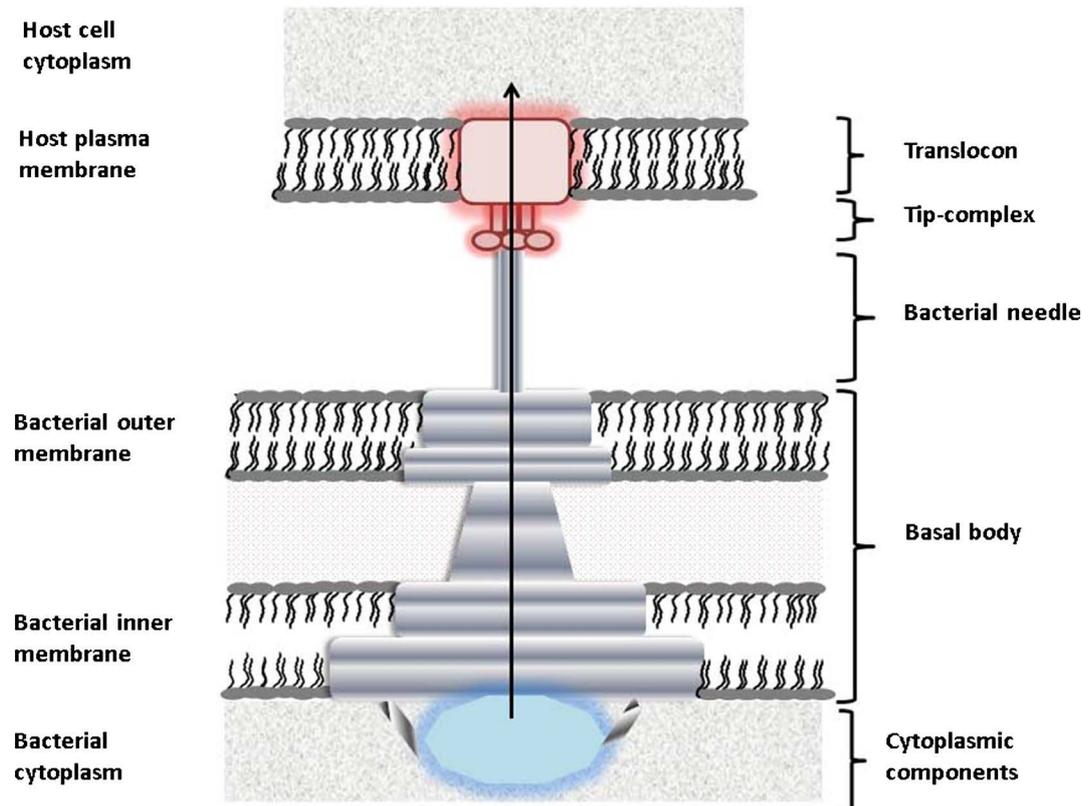


Figure 1. Illustration of type III secretion system (T3SS) in *C. rodentium*. The T3SS consists of a complex apparatus that actively delivers bacterial effector proteins into the cytoplasm of a host cell. The basal body spans the bacterial membranes and a needle-like syringe extends from the surface which is capped by a tip complex. Upon host cell contact, a translocon is inserted in the host cell membrane and forms a pore. Bacterial virulence proteins are then selectively secreted through the syringe into the host cell, where they manipulate host cell functions essential for subsequent pathogenicity.

2.1. Virulence Factors of *C. rodentium*

The type III secretion system is programmed by a cluster of genes recognized as locus of enterocyte effacement (LEE), a conserved pathogenicity island consisting of 35.6 kb [24]. The LEE pathogenicity island consists of over 40 genes which are organised in to five operons including LEE1, LEE2, LEE3, LEE4 and LEE5 [24] [25] [26]. LEE encodes several structural components of T3SS, effectors, translocators and several other proteins (Figure 2) [27] [28]. One important effector protein is Tir (translocated intimin receptor) a bacteria derived receptor, which following translocation binds to the host epithelial cell and interacts with intimin, the bacterial outer membrane protein, thereby facilitates anchorage of bacteria to host cell, leading to pedestal formation [29] (Figure 3). The intimins are encoded by *eae* genes that are extremely conserved in N-terminal regions, however, display substantial heterogeneity at the C-termini [30]. Five different intimin types α , β , γ , δ and ϵ have been recognized [31] [32]. Intimin α and intimin β are expressed mainly by strains pertaining to EPEC clones 1 and 2, correspondingly, whereas intimin γ is expressed by enterohaemorrhagic *E. coli* (EHEC) serotype O157:H7 and intimin δ by EPEC O86:H34 [33].

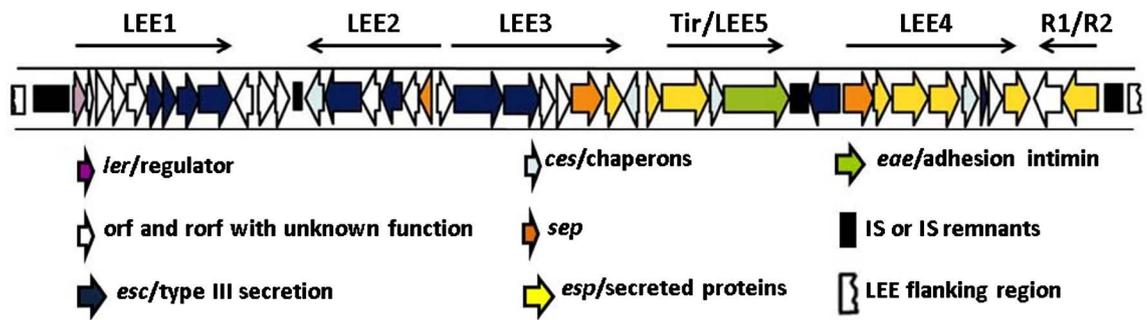


Figure 2. Genetic assembly of *C. rodentium* LEE. The orientation of each gene is shown by the direction of the arrow. The different locations of the *rorf1* (*r1*) and *rorf2* (*espG/r2*) genes in *C. rodentium* LEE, as well as the association of several IS's or IS remnants with the *C. rodentium* LEE. The major operons encoded by the LEE (LEE1, -2, -3, and -4, Tir, and R1/R2) and their transcriptional directions are shown and adapted from reference [25].

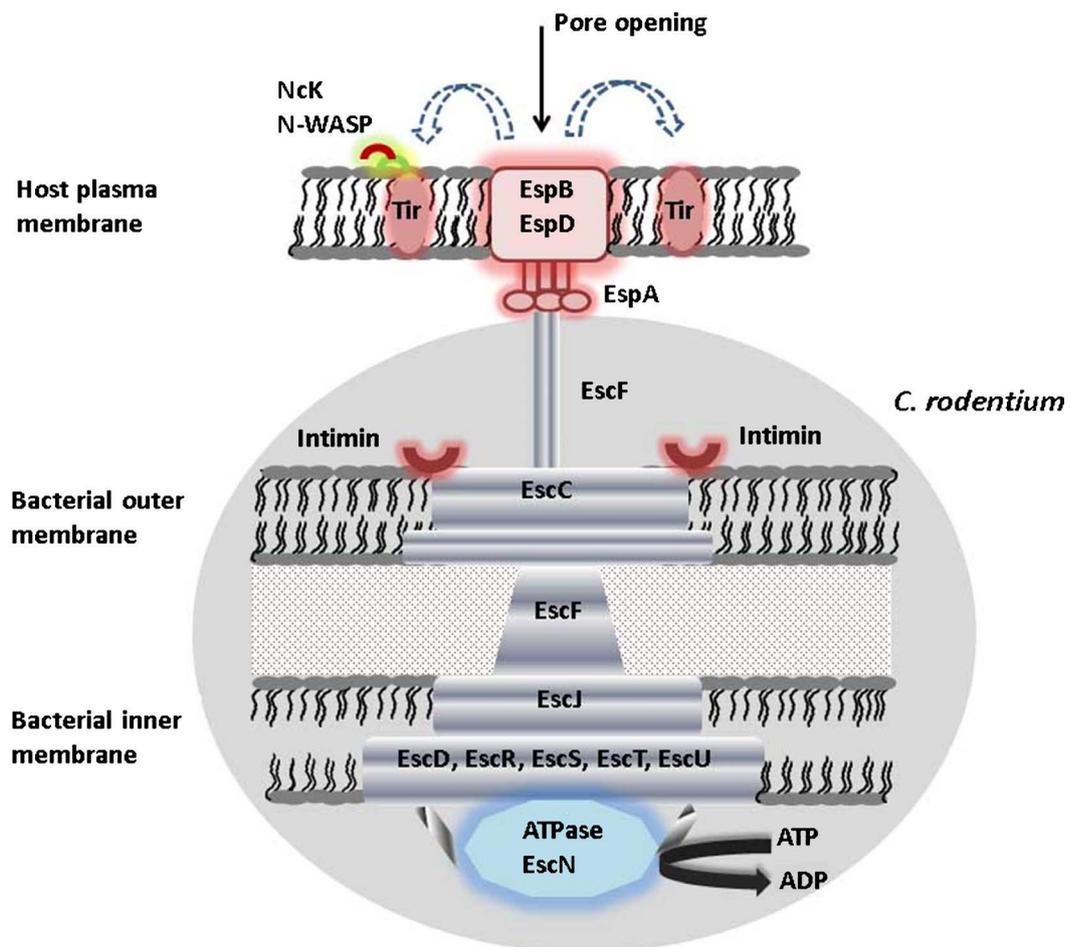


Figure 3. Translocation of *C. rodentium* secreted proteins occurs through a LEE-encoded type III secretion system that spans the inner and outer membranes of the bacteria as well as that of the host cell. EscJ is the inner membrane ring which forms the platform for all other Type III secretion components. EspA-containing surface organelles form a filamentous tube for the translocation into the host cell cytosol. EspB and EspD are then inserted into the host cell membrane, form a pore structure in the plasma membrane enabling the passage of bacterial effectors, such as Tir. Tir serves as a receptor for the outer membrane ligand, intimin. Tir and intimin interaction initiates a signal transduction cascade that aids in the recruitment of actin to form attaching and effacing (A/E) pedestals beneath the adherent bacteria. Tir molecules recruit Nck leading to the activation of N-WASP and strong actin polymerization.

Upon entry to the host cells, Tir is tyrosine phosphorylated, which recruits non-catalytic region of tyrosine kinase adaptor protein Nck [34] [35] [36] [37]. Nck binds to the phosphorylated tyrosine and this in turn triggers the recruitment of nucleation promoting factor N-WASP (neural Wiscott-Aldrich syndrome protein) and actin-regulated protein Arp2/3 complex, resulting in host actin rearrangement [38] [39] [40]. Tir triggers localized actin polymerization by another two different pathways. After translocation, LEE and non-LEE effectors contribute to the impediment of several signalling pathways in the host cell, including actin polymerization, tight junction integrity, endosomal trafficking, apoptosis, phagocytosis and innate immune responses, as well as epithelial cell shedding and detachment [41].

Besides Tir, LEE encodes several secreted translocators: EspA, EspD, EspB, EspF, EspG, EspH, EspZ, which are entirely translocated into the host cells and are involved in modulating host cytoskeleton leading to the manifestation of disease [4] [42] [43]. A/E pathogens secrete numerous LEE-encoded regulatory proteins, Ler, GlrA and GlrR, which exhibit a significant role in the transcriptional regulation of LEE and several non-LEE virulence determinants [44] [45] [46]. Moreover, RegA adjusts LEE expressions through upregulating *grlR/A* transcription [47]. There are several effectors that are not secreted and translocated by the LEE-encoded T3SS including prophages and insertion sequences. They comprise the EspI/NleA, an indispensable protein for entire virulence of *C. rodentium* [48] [49] [50] and binds host PDZ-domain proteins [51]; EspB, essential for intimate attachment and signal transduction (Figure 3) [52]; EspJ, which display a negligible part in enteric colonization [53]; and EspG, stimulates the dissociation of microtubules beneath adherent bacteria [54]. In addition, two non-LEE encoded proteins, NleB to NleH, are found in *C. rodentium* and they are mostly produced by the LEE-encoded T3SS [49] [55]. Among these, NleC and NleD have been recognized to be translocated into host cells [56]. NleB1 binds to host cell death domain encoding proteins, diminishes the signalling of a death receptor and thereby disrupting a major antimicrobial host response [57].

Other than the genes for *rorf1* and *rorf2/espG* and several insertion sequences (IS) and IS remnants, both the LEE of *C. rodentium* and that of EPEC and EHEC shares all 41 ORFs and the linear gene sequences (Figure 2) [25]. This suggests that the LEE encoded pathogens has a mutual evolutionary origin and reciprocal function which supports the use of *C. rodentium* as an animal model to study A/E pathogenesis.

2.2. Disease Progression of *C. rodentium*

Similar to EPEC and EHEC, *C. rodentium* infection encompasses three distinct phases: 1) an initial colonization phase specially facilitated by bacterial effector proteins, 2) an acute phase characterized by colonic hyperplasia with the initiation of diarrhoea in severe cases, 3) a convalescent phase manifest as the clearance of bacteria and the prevention of further invasion.

During the first week after inoculation, *C. rodentium* colonizes the brush border microvilli and higher numbers of bacteria are seen closely adherent to the mucosal epithelial cells (Figure 4) [59] [60]. Acute phase of infection follows over the subsequent 2 weeks when the levels of bacteria peak around $>10^9$ c.f.u per gram of tissue in the colon [16] and the bacteria induce a profound hyperplasia of colonic mucosa with the development of secretory diarrhoea [61]. At the time of peak hyperplastic phase, the organism can no longer be isolated from the intestines and the infected mucosa are thickened markedly. Convalescent phase of infection comes following 4 weeks and above, for the period of which the reactive epithelial hyperplasia to clinical diarrhoea get resolved and colonic mucosa appears normal [13] [62].

2.3. Immune Defense against *C. rodentium*

Most of the existing knowledge on the immune response and its relation with pathology has been expanded using mice with the targeted ablations of various immune components. Innate immune response as well as adaptive immune response appears to control mucosal defence against *C. rodentium* [63].

2.3.1. Role of Adaptive Mucosal Immune Responses

The concept of exploring mice with deficiencies in immune components first came from the study that colonic mucosa of infected mice contained large infiltrates of CD4⁺ T cells with a helper T cell 1 cytokine response [64]. Substantial mortality was observed in mice deficient in CD4⁺ T cells or TCR $\alpha\beta$ ⁺ T cells [59]. Mice lacking CD4 showed a survival limit of two weeks and exhibited 100%

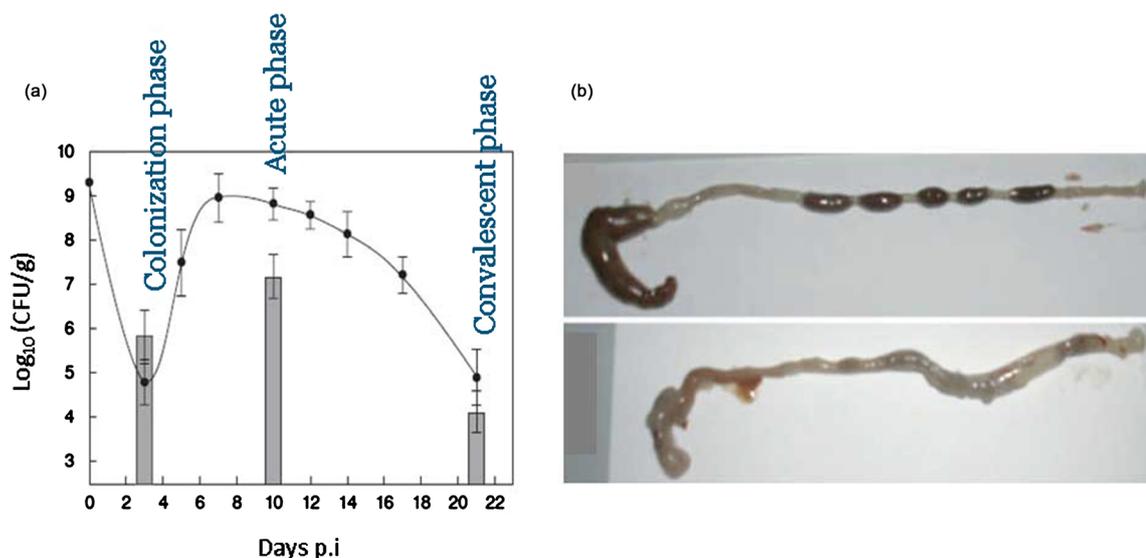


Figure 4. Infection kinetics in C57BL/6 mice infected with *C. rodentium*. (a) *C. rodentium* bacterial burden at three distinct phases of infection: A colonization phase which persists from day 3 to day 5 after infection, an acute phase manifest as diarrhoea and host inflammatory responses and a convalescent phase during which the host clears pathogen (partially adapted from [58]); (b) The image here represents the gastrointestinal tract isolated from a normal (upper one) and an infected mouse (lower one). Infection results in empty and retracted caecum and a thickened colon devoid of normal stool (partially adapted from [12]).

mortality. However, depletion of CD8⁺ T cells or TCR $\gamma\delta$ T cells did not adversely affect survival of infection and played a minor role in surviving the acute phase of infection. Two studies [63] [65] [66] separately demonstrated the vital significance of B cells for the protective immunity against *C. rodentium*. Mice lacking mature B cells (μ MT mice) failed to mount early inflammatory response at 2 weeks and could not lessen bacterial load or clear bacterial colonization over a prolonged period [63] [65] [66]. RAG1-deficient mice in which both B and T cells were absent displayed chronic intestinal colonization and more severe colonic damage and these mice were unable to clear infection and died after 3-4 weeks [59] [63] [67].

To analyse the potential of secretory antibodies in bacterial clearance, mice with selective deficiencies for IgA, IgM or IgG were used [65]. Both IgA- and IgM- deficient mice had been found to develop effective immunity against a secondary challenge and played a negligible role in controlling *C. rodentium* infection. However, mice deficient in IgG antibodies lost the ability to develop robust protective response against secondary challenge. Thus host defense against *C. rodentium* was dependent on IgG antibodies but did not require secretion of IgA or IgM [65]. A comparative analysis of the specific ablation of different adaptive immune components is summarized in **Table 1**.

Simmons and co-workers investigated *C. rodentium* infection in IFN γ -deficient and IL-12 deficient mice. IFN γ -deficient mice had higher bacterial numbers and

Table 1. Summary of the effects of selective ablation of adaptive immune components on *C. rodentium* infection in mice.

Mouse models	Effects of ablation of specific adaptive immune components on <i>C. rodentium</i> infection	Refs
Mice lacking CD4 ⁺ T cells or TCR $\alpha\beta$ + T cells	A survival limit of two weeks and exhibited 100% mortality	[59] [64]
Mice lacking CD8 ⁺ T cells or TCR $\gamma\delta$ T cells	Do not adversely affect survival of infection	[59]
Mice without mature B cells (μ MT mice)	Cannot lessen bacterial load or clear bacterial colonization over a prolonged period	[63] [65] [66]
RAG1-deficient mice (both B and T cells are absent)	Develop chronic intestinal colonization and unable to clear infection and die after 3-4 weeks	[59] [63] [67]
IgA- and IgM- deficient mice	Develop effective immunity against a secondary challenge and play a negligible role in controlling <i>C. rodentium</i> infection.	[65]
Mice deficient in IgG antibodies	Lose the ability to develop protective response against secondary challenge.	[65]
IFN γ -deficient mice	Higher bacterial numbers and enhanced mucosal thickening in colons and cannot clear infection until day 28	[68]
IL-12 deficient mice	Elicit higher bacterial numbers for the first 3 weeks of infection and eventually clear infection by day 35	[30]
Mice lacking IL-22	Display systemic bacterial load and enhanced epithelial hyperplasia and mortality range up to 100% within the first two weeks of infection	[69]
Treg deficient mice (DEREG mice)	Diminished bacterial clearance, systemic dissemination of bacteria with compromised Th17 immune response accompanied by less inflammation-associated pathology	[70]
IL-10 ablated mice	Resolve infection earlier than wild-type mice with less infection associated colitis	[71]

enhanced mucosal thickening in their colons and could not clear infection until day 28 [68]. Alternatively, IL-12 deficient mice had been shown to elicit higher bacterial numbers for the first 3 weeks of infection and eventually cleared infection by day 35 [30]. Interleukin-22 (IL-22) has been identified as an essential cytokine for mediating protection against *C. rodentium* infection. While compared to wild-type mice, mice lacking IL-22 infected with *C. rodentium* displayed systemic bacterial load and enhanced epithelial hyperplasia. In multiple repeat experiments, the mortality of IL-22 knockout mice ranged from 80% to 100% within the first two weeks after infection [69]. Administration of Reg3 γ , an antimicrobial peptide to IL-22 knockout mice controlled infection.

During infection, CD4⁺ Th17 cell subsets were particularly amplified in Peyer's patches (PP) but were unaltered in mesenteric LNs [70]. The differentiation of Th17 cells in PP were dependent on the inflammatory cytokine IL-6 as treatment with anti-IL-6 antibodies reduced Th17 cells and exacerbated clinical manifestation of colitis. Moreover, following anti-IL-6 antibody treatment, there was a reduction of IL-22 mRNA expression in the small intestine during infection but had no effect on IgA production by B cells [70].

Symonds and co-workers demonstrated an elevated FoxP3 mRNA expression in the distal colon at all stages of infection with *C. rodentium*. Also, *C. rodentium* infection exhibited an up-regulation of IL17 mRNA expression [58]. Wang and co-workers investigated role of Treg during *C. rodentium* infection using DEREK mouse model. Depletion of Treg by diphtheria toxin led to a diminished bacterial clearance and systemic dissemination of bacteria. Also, Th17-associated immune response was compromised following Treg-depletion, with less inflammation-associated pathology in the colons of Treg-depleted mice. Treatment with Anti-IL2 in depleted mice retained Th17 induction, suggesting that Treg induced a protective Th17 response by intake of local IL-2 [71]. IL-10 was found dispensable in controlling inflammation as IL-10 ablated mice resolved infection earlier than wild-type mice and had less infection associated colitis [72]. In addition, following infection, IL-27 was produced which subsequently suppressed Th17 in vitro and thus play role in anti-inflammatory circuit in the absence of IL-10. The neutralization of IL-27 led to pronounced colitis in mice lacking IL-10 suggesting that IL-10 enacts a minor part in the bacterial clearance whereas IL-27 might be an important cytokine for attenuation of inflammation [72].

Th22 cells were found to be important in the mucosal anti-microbial host defense against *C. rodentium*. Basu and co-workers [73] demonstrated that *C. rodentium* induced a wave of IL-22 producing ILCs and CD4⁺ T cells were each critical to host protection during infection. Though the IL-22 production by ILCs was strictly IL-23 dependent, IL-22 production by CD4 cells was not IL-23 dependent rather the production was dependent on IL-6 and transcription factors T-bet and AhR. Also IL-22 producing CD4 cells (Th22) were more effective in host protection than Th17 cells. IL-17 was found to be important in host defense against *C. rodentium* [74]. The bacterial burden in the colon after infection

with *C. rodentium* showed similar increases in IL-17f^{-/-}, IL17a^{-/-}, and IL-17a^{-/-}IL17f^{-/-} mice, indicating that deficiency of just one of the IL-17 proteins resulted in full susceptibility to infection. However, splenomegaly and colon hypertrophy, which were associated with severe colonic inflammation, were more pronounced in IL-17f^{-/-} mice than in IL-17a^{-/-} mice suggesting that IL-17f was more important than IL-17a in protecting colonic epithelial cells from the pathogenic effects of this bacterium.

2.3.2. Role of Innate Mucosal Immune Responses

There are several innate immune components which perform a vital role in mucosal homeostasis and in antimicrobial immunity. An augmented pathology was observed in mice lacking Toll-like receptor 2 (TLR2) due to an impaired epithelial barrier [75]. Mice lacking the signalling adaptor MYD88, a myeloid differentiation primary response protein 88, which is essential for signalling by the majority of TLRs [76] [77] had greater bacterial loads both in the colon and in peripheral tissues as the bacteria penetrated deeply into colonic crypts compared to WT mice. Moreover, they suffered from severe colitis and death after infection. The innate immune receptor type-I interleukin-1 receptor (IL-1R), utilizing MyD88 signalling pathway protected mice from severe damage caused by *C. rodentium* [78]. IL-1R deficient mice exhibited increased susceptibility to tissue damage comparable to that of MyD88 knockout mice. Yet, distinct from MyD88 knockout mice, mice deficient in IL-1R did not display amplified pathogen burdens in the colon. In another study, Khan and co-workers exhibited that the bacteria triggered TLR4 and prompted NF- κ B nuclear translocation which was dependent on TLR4. Deficiency of TLR4 decreased tissue pathology and inflammatory cell infiltration in gut. Unexpectedly, dissemination of bacteria through colon was hindered in mice lacking TLR4, while the extent of infection was unaffected, suggesting that TLR4-mediated responses were eventually maladaptive to the host [79].

Liu and co-workers demonstrated the biological function of inflammasomes in immune response against *C. rodentium*. Mice deficient in inflammasome components Nlrp3, Nlrc4, and caspase-1 were hyper susceptible to *C. rodentium* induced intestinal inflammation due to impaired production of IL-1 β and IL-18 [80]. However, these deficient mice exhibited only mild defects and none of these mice died after infection, indicating that inflammasome is not essential for mice survival after *C. rodentium* infection. In addition, IL-1 β ^{-/-} and IL-18^{-/-} mice suffered from increased bacterial burdens and had severe histopathology. Therefore, Nlrp3 and Nlrc4 inflammasome-mediated IL-1 β and IL-18 response contributed a significant role in host protection against *C. rodentium* [80] [81]. In another study, Kim and co-workers characterized the role of the intracellular Nod-like receptor family members Nod2 in protection against *C. rodentium* infection [82]. Nod2^{-/-} mice displayed diminished intestinal clearance to *C. rodentium*. The enhanced bacterial load was due to impaired secretion of chemokine ligand 2 (CCL2) from colonic cells and subsequent inflow of monocytes. Fur-

thermore, IL-12, a cytokine produced by monocytes triggered Th1 immunity vital for bacterial clearance. The adoptive transfer experiments established the significant contribution of Ly6C^{hi} monocytes in the clearance of bacteria in vivo [82]. **Table 2** summarizes a comparative analysis of the specific ablation of different innate immune components.

Mice lacking the p50 subunit of the NF- κ B transcription factor, a nuclear factor kappa B, had reduced ability to clear *C. rodentium* infection [83]. Also a continued bacterial load was reported in mice deficient in p38 α , a mitogen-activated protein kinase (MAPK) in intestinal epithelial cells [84]. Interestingly, these animals exhibited no apparent histological lesions, however, failed to recruit CD4⁺ T cells and had impaired chemokines expression. Thus, p38 α in IECs by employing immune cells and adjusting chemokine expression played a part to the host protective immune responses. CXCL9, an ELR (glutamic acid-leucine-arginine) motif chemokine had direct antimicrobial potential against *C. rodentium* and defended crypts from bacterial dissemination. Blockade of this antimicrobial activity by anti-CXCL9 antibodies escalated host exposure to *C. rodentium* infection

Table 2. Summary of the effects of selective ablation of innate immune components on *C. rodentium* infection in mice.

Mouse models	Effects of ablation of specific innate immune components on <i>C. rodentium</i> infection	Refs
Mice lacking Toll-like receptor 2 (TLR2)	An augmented pathology due to an impaired epithelial barrier	[75]
Mice lacking MYD88	Have greater bacterial loads in colon and peripheral tissues and suffer from severe colitis and death	[76] [77]
IL-1R deficient mice	Increased susceptibility to tissue damage but do not display amplified pathogen burdens in colon.	[78]
Deficiency of TLR4	Decreased tissue pathology and inflammatory cell infiltration in gut. While the extent of infection is unaffected, dissemination of bacteria through colon is hindered	[79]
Mice deficient in Nlrp3, Nlr4, and caspase-1	Hyper susceptible to <i>C. rodentium</i> induced intestinal inflammation. However, exhibit only mild defects and do not die after infection	[80] [81]
IL-1 β ^{-/-} and IL-18 ^{-/-} mice	Increased bacterial burdens and severe histopathology.	[80] [81]
Nod2 ^{-/-} mice	Diminished intestinal clearance to <i>C. rodentium</i> . due to impaired secretion of CCL2 from colonic cells	[82]
Mice lacking the p50 subunit of NF- κ B	Reduced ability to clear <i>C. rodentium</i> infection.	[83]
Mice deficient in p38 α	A continued bacterial load with no apparent histological lesions, however, fails to recruit CD4 ⁺ T cells and impaired chemokines expression.	[84]
Ablation of specific macrophage/monocyte compartment	Neither cell type is essential to trigger immunity	[85]
Mice lacking PSGL-1 and P, E and L-selectin	Mice defective in PSGL-1 and P-selectin suffer morbidity, extensive inflammatory responses and augmented bacterial burden, however, mice defective in either E or L-selectin do not exhibit severe infection	[86]
Mice lacking β_7 integrin	Efficiently control infection and clear bacteria 5-6 week after inoculation	[59]
Mice deficient Muc2	Susceptible to the <i>C. rodentium</i> -induced colitis and display quick weight loss and exhibit 90% mortality	[87] [88]

with noticeable bacterial dissemination, augmented bacterial titre, and deteriorated tissue pathology [89]. Surface lymphotoxin expression on group 3 innate lymphoid cells (ILC3s) is critical for early immune responses against *C. rodentium* [90] [91]. LT aids in IL-22 secretion by intestinal ILCs. Blocking of LT β R signaling rapidly diminished intestinal IL-22 production after *C. rodentium* infection [91]. In addition, stimulating LT β R signaling induced IL-22 pathway in LT-deficient mice. LT-beta receptor (LT β R) signaling in intestinal epithelial cells was essential for recruitment of neutrophils to the site of infection through secretion of CXCL1 and CXCL2 chemokines. In contrast, surface LT produced by adaptive B and T cells was dispensable for protection against gut bacterial infection [90].

The function of macrophages and monocytes during *C. rodentium* infection was investigated using ablation of specific macrophage/monocyte compartment during infection. Although neither cell type was essential to trigger immunity, monocytes and macrophages played a role by secreting IL-12, which prompted Th1 polarization and IFN- γ secretion. Thus, monocytes and macrophages contribute in *C. rodentium* immunity by secreting cytokines that direct T cell polarization [85].

To outline the function of selectins and their ligands during *C. rodentium* infection, Kum and co-workers investigated infection in mice lacking PSGL-1, a P-selectin glycoprotein ligand-1 and P, E and L-selectin [86]. Mice defective in PSGL-1 and P-selectin suffered morbidity, extensive inflammatory responses and augmented bacterial burden, however, mice defective in either E or L-selectin did not exhibit severe infection. Also, intestinal inflammation and recruitment of inflammatory cells *i.e.*, neutrophils and macrophages were significantly diminished in P-selectin defective mice which received blocking antibodies to ICAM-1 or LFA-1, suggesting that these adhesion molecules can counter-balance the defect in selectins during leucocyte recruitment [86]. Mice lacking β_2 integrin efficiently controlled infection and cleared bacteria 5-6 week after inoculation [59].

Mice deficient in main intestinal mucin, Muc2, which have an altered intestinal mucus layer, were more susceptible to the *C. rodentium*-induced colitis and displayed quick weight loss and exhibited about 90% mortality due to a closer interaction of intestinal microbes with the epithelial barrier [87] [88]. *Muc2*^{-/-} mice had 10 - 100 fold increased *C. rodentium* load, maximum of which were closely attached to the mucosa in colon. FITC-Dextran administration exhibited considerably exacerbated disruption in intestinal barrier integrity in *Muc2*^{-/-} mice, with explicit bacterial translocation into the colonic mucosa [87] [88].

2.4. Role of Probiotics and Antibiotic Administration

Probiotics, a combination of live microorganisms attenuated infection with *C. rodentium* in adult mice and provided a protective role in *C. rodentium* induced death in neonatal mice [92]. In one study, it had been shown that probiotic

mixture exhibited inhibitory role on the growth of *C. rodentium*. Mice that were administered live probiotics containing a mixture of *Lactobacillus rhamnosus* and *L. acidophilus* stayed healthy. Pretreatment of mice with probiotics restored colonic integrity and lessened both hyperplasia and inflammatory-cell infiltration in colon [93]. In a recent study, Collins *et al.* demonstrated that probiotics such as *Lactobacillus acidophilus*, *L. rhamnosus*, and *Lactobacillus helveticus* administered daily in the form of fermented dairy products (FDPs) lessened *C. rodentium* induced colonic hyperplasia and stopped the loss of significant bacterial genera that might lead to disease pathology. However, the FDPs did not result in any noteworthy reduction in *C. rodentium* colonization when estimated by bacterial load [94].

Metronidazole pretreatment augmented exposure to *C. rodentium*-induced colitis compared to that of untreated mice 6 days postinfection and resulted in a diminished number of *Porphyromonadaceae* and amplified population of *lactobacilli* [95]. Metronidazole treatment resulted an impaired goblet cell function, decreased Muc2 secretion, a major component of intestinal secretory mucin and thinning of inner mucus layer, resulting in microbially induced immune activation prior to disease induction. Perturbation of the microbiota with metronidazole resulted augmented attachment of bacteria to the intestinal epithelium, resulting in a severe form of *C. rodentium*-induced colitis in mice [95].

2.5. Limitations of *C. rodentium* Model

A limitation to the study of *C. rodentium* infection model is the absence of antigen-specific tools with which to characterize the fate and function of the pathogen/antigen-specific response during infection [96]. The only means that are currently available to address this limitation include transgenic strains of *C. rodentium* that express OVA or GFP [96] [97] [98]. Another probable limitation to study this pathogen could be the loss of antibiotic sensitivity of *C. rodentium* due to the development of worldwide emergence of multi-resistant strains [19]. However, the likelihood of this loss is occasional due to the germ-free condition of the animal houses.

3. Concluding Remarks

EPEC and EHEC are the leading cause of diarrhoea in human, affecting children and adults in both developing and developed countries. *C. rodentium* is an enteric murine pathogen that mimics virulence factors of human EPEC and EHEC and forms comparable attaching and effacing lesions, as a central mechanism of tissue targeting, virulence factors and infection in mice. As a result of this association with other important inflammatory diseases, and that there are cases of more than a million deaths each year from EPEC and EHEC, the knowledge about the pathophysiology of *C. rodentium* infections and following infection how the host immune system responds to it is of immense significance to understand its subsequent function during these inflammatory diseases.

This review comprehensively covers the salient features of recent discoveries related to *C. rodentium* virulence, epithelial hyperplasia, innate and adaptive immune responses, and the pathophysiology of diarrhoea. It is acknowledged that EPEC and EHEC can be modelled efficiently in mice. Murine *C. rodentium* is a well characterised model of diarrhoeal disease as the molecular, cellular, pathophysiological aspects of the disease have been well studied. Therefore, *C. rodentium* represents an excellent model in which to study the innate and adaptive immune components. We believe that the advances that have been included in this review will give a comprehensive insight to combat the acute diarrhoeal illness in human. Nevertheless, once again *C. rodentium* has been proved to be a useful *in vivo* model for studying pathogenesis of secretory diarrhoeal diseases/gastrointestinal pathogen and for preventive/mucosal vaccinations and therapeutic approaches.

Conflicts of Interest

The authors declare no conflict of interest that could be perceived to bias the work.

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