Activation of p38α in T Cells Regulates the Intestinal Host Defense against Attaching and Effacing Bacterial Infections

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Intestinal infections by attaching and effacing (A/E) bacterial pathogens cause severe colitis and bloody diarrhea. Although p38α in intestinal epithelial cells (IEC) plays an important role in promoting protection against A/E bacteria by regulating T cell recruitment, its impact on immune responses remains unclear. In this study, we show that activation of p38α in T cells is critical for the clearance of the A/E pathogen Citrobacter rodentium. Mice deficient of p38α in T cells, but not in macrophages or dendritic cells, were impaired in clearing C. rodentium. Expression of inflammatory cytokines such as IFN-γ by p38α-deficient T cells was reduced, which further reduced the expression of inflammatory cytokines, chemokines, and antimicrobial peptide by IECs and led to reduced infiltration of T cells into the infected colon. Administration of IFN-γ activated the mucosal immunity to C. rodentium infection by increasing the expression of inflammatory genes and the recruitment of T cells to the site of infection. Thus, p38α contributes to host defense against A/E pathogen infection by regulating the expression of inflammatory cytokines that activate host defense pathways in IECs. The Journal of Immunology, 2013, 191: 2764–2770.

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fection by attaching and effacing (A/E) bacterial pathogens, including enteropathogenic Escherichia coli (EPEC) and enterohemorrhagic E. coli (EHEC), is a common and potentially serious cause of gastroenteritis around the world. EHEC O157:H7 (E. coli O157:H7) infection causes severe colitis and bloody diarrhea due to the production of shiga-like toxins, whereas EPEC causes diarrhea in millions of children in developing countries. Because EPEC and EHEC are human specific and do not infect mice efficiently, infection of the natural mouse pathogen Citrobacter rodentium is a commonly used model, which has provided information about the A/E bacterial pathogenesis and the host immune response (1). Both innate and adaptive immune responses contribute to host defense against C. rodentium infection (2–6). TLRs have been demonstrated to play a major role in the recognition of C. rodentium infection and in initiating the inflammatory immune responses (4, 7). Additionally, intracellular innate NOD-like receptors participate in host defense by inducing Th1 and Th17 and responses in the gastrointestinal tract (8, 9). Meanwhile, CD4+ T cells are the critical mediators for the adaptive immune response to C. rodentium in the murine colonic mucosa (3, 10), and a Th1/Th17-mediated response is associated with host defense against C. rodentium infection (6, 11). Additionally, certain cytokines such as IFN-γ and TNF-α from lymphocytes play a critical role in host defense in C. rodentium infection (12, 13). These cytokines stimulate not only the innate immune response of the infected epithelial cells, but also the inflammatory phenotypes of lymphocytes (11).

p38α is a member of the serine-threonine MAPK family and regulates numerous biological processes, including immune responses and inflammation (14–16). p38α-mediated expression of proinflammatory cytokines and chemokines is initiated by TLR responses in innate immune cells (17), and p38α also plays an important role in the pathology of skin and gut inflammation (18–20). p38α regulates the development of T cells in thymus, differentiation of naïve T cells into Th effector cells, and production of cytokines that contribute to inflammation and host defense (16, 21–23).

We previously demonstrated that expression of inflammatory cytokines and chemokines was reduced, and infiltration of T cells was impaired in the colon of C. rodentium–infected intestinal epithelial cell (IEC)–specific p38α-deficient mice, which resulted in the failure of bacterial clearance (24). However, the role of p38α expression in immune cells in controlling the host response to A/E pathogen infection is unknown. In this study, we generated immune cell–specific p38α-deficient mouse strains and investigated the role of p38α in controlling the immune response against C. rodentium infection. We observed that p38α expression by T cells is critical for host clearance of C. rodentium by producing the inflammatory cytokines that activate IEC defense mechanisms.

Materials and Methods

Mice

p38α<sup>−/−</sup> mice were described previously (17). To generate macrophage-, dendritic cell (DC)–, or T cell–specific p38α-deficient mice, p38α<sup>−/−</sup> mice

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were bred with Ly5.1, CD11c, or Lck promoter-driven Cre transgenic mice (The Jackson Laboratory, Bar Harbor, ME). C57BL/6J wild-type mice were obtained from Institutional Breeding Colony at the Scripps Research Institute. Animal studies were performed using sex-matched 8- to 10-week-old mice and conducted according to the guidelines and approval of the Institutional Animal Care and Use Committee.

**Bacterial infection and bacterial Ag preparation**

*C. rodentium* strain DBS 100 (American Type Culture Collection, Manassas, VA) in a volume of 200 μl (2 × 10^7 CFU) was orally inoculated into each mouse. Colon biopsies were collected at 8 h. IFN-γ (10 μg per mouse; R&D Systems) was injected i.p. at the indicated time points after *C. rodentium* infection. *C. rodentium* lysate was prepared, as previously described (24, 25).

**CFU count, colon tissue collection, and cell isolation**

To assess the level of *C. rodentium* infection, in colonic tissues, a distal piece (~1 cm) of colon was removed, weighed, and homogenized in sterile PBS. Homogenates were serially diluted in PBS and plated on MacConkey agar.

The number of colonies was counted after 18 h of incubation at 37°C. To obtain RNA of colon tissues, a piece of colon (~0.5 cm) was collected and kept in RNAlater (Qiagen) at −80°C until the RNA preparation. IECs and lamina propria (LP) lymphocytes were obtained as previously described (24).

Briefly, the colon was removed and opened longitudinally, and then washed with ice-cold PBS to remove debris and mucus. The tissue was cut into small pieces (~1 cm) and further incubated at 37°C for 15 min under gentle shaking in HBSS supplemented with 5 mM EDTA and 2% FBS. The epithelial cells in the supernatant were collected spun down at 150 × g for 5 min. The cells pellets were resuspended in 40% Percoll solution and spun down again. The epithelial cells at the top layer were collected. The purity was assessed by staining the cells with epithelial cell–specific markers anti-cytokeratin-18 (C-04; Santa Cruz Biotechnology) and anti-EpCAM (G8.8; eBioscience) (>95% purity). The remaining tissue pellets after the gentle shaking in HBSS with 5 mM EDTA and 2% FBS were further incubated at 37°C for 2 h in RPMI 1640 medium containing 20% FBS, 2 mg/ml collagenase type III (Worthington Biochemical), and 15 mg/ml DNase I (Roche). The cells were further washed in HBSS and passed through a 40 μm cell strainer to harvest LP lymphocytes.

**Histological analysis**

To compare the degree of inflammation, colon tissues were fixed in 10% formalin, and then embedded in paraffin for microscopic analysis. The histological scoring was assessed to determine the degree of inflammatory cell infiltration and tissue damage (26). The cell infiltration score was defined as a scale of 0–3 of inflammatory cell infiltration (0, no or occasional inflammatory cells in the LP; 1, slightly increased number of inflammatory cells; 2, moderate infiltration of inflammatory cells; 3, extensive infiltration of inflammatory cells), and the histological tissue damage score was determined (0, no damage; 1, mild hyperplasia with superficial epithelial injury; 2, moderate hyperplasia, with focal erosions; 3, severe hyperplasia with multifocal erosions).

**Immunohistochemistry**

Immunostaining of *C. rodentium* and CD4 T cells was performed as follows. Paraffin section slides were deparaffinized and rehydrated prior to Ag retrieval by boiling in 10 mM sodium citrate buffer (pH 6.0). Sections were blocked in blocking buffer (3% BSA and animal-free blocker [Vector Labs, Burlingame, CA]) and stained with rabbit anti–*C. rodentium* Ab, followed by Alexa Fluor 488 anti-rabbit IgG (Molecular Probe, Eugene, OR), or stained with FITC-conjugated anti-mouse CD4 (BioLegend, San Diego, CA). Slides were counterstained with VECTASHIELD mounting media with DAPI (Vector Labs, Burlingame, CA) prior to visualization.

**Preparation and stimulation of T cells**

T cells were purified using Pan T Cell Isolation Kit II (Miltenyi Biotec, Auburn, CA) from spleen. To induce T cell activation, cells were seeded on the plates coated with anti-CD3/CD28 Abs. After 3 d, culture supernatants were collected to determine IL-2 and IFN-γ levels by ELISA. Cell proliferation was tested using CFSE cell proliferation kit (Invitrogen). The cells were labeled with CFSE and cultured in the plates coated with anti-CD3/CD28 Abs for 3 d. Degree of proliferation was measured using flow cytometer, according to the manufacturer’s protocol.

**Ex vivo colon culture and cytokine measurement**

Colon fragments (~1 cm) were obtained aseptically and weighed. The pieces were washed three times in ice-cold PBS and incubated in DMEM supplemented with 10% FBS and antibiotics for 24 h (24, 25). Culture supernatants were obtained, and cytokine levels were measured by ELISA.

**Preparation of bone marrow–derived DCs**

Bone marrow cells were obtained and cultured in RPMI 1640 supplemented with 10% FBS and GM-CSF (20 ng/ml) for 6 d.

**Preparation of FITC-conjugated *C. rodentium* and analysis of phagocytic activity of macrophages and DCs**

Labeling of *C. rodentium* and phagocytosis analysis was as previously described (27). Cultured *C. rodentium* was labeled with 1 mg/ml FITC solution in PBS for 15 min. Peritoneal macrophages from control or macrophage-specific p38α-deficient mice, or bone marrow–derived DCs from control or DC-specific p38α-deficient mice were cocultured with FITC-conjugated *C. rodentium* for 6 h. Cells were washed and harvested in ice-cold PBS. DCs were stained with anti-CD11c PE Ab. Intracellular level of FITC-labeled *C. rodentium* was measured by flow cytometry.

**Flow cytometry of intracellular cytokine and surface marker expressions**

Isolated cells from LP were treated with brefeldin A (10 μg/ml), PMA (50 ng/ml), and ionomycin (1 μg/ml) for 4 h before intracellular cytokine staining of IL-17 and IFN-γ. Intracellular staining of cytokines was performed using Cytofix/CytoPerm Fixation/Permeabilization Solution kit (BD Biosciences). LP and MLN cells were suspended in FACS buffer and incubated with the indicated Abs for FACS analysis. Fc Block (anti-CD16/CD32), anti-CD4 allophycocyanin, anti–IL-17A FITC, anti–IFN-γ PE, anti-CD11c allophycocyanin, anti–TNF-α FITC, anti–MHCII PE, anti–CD80 PE, and anti-CD86 FITC (eBiosciences) Abs were used as indicated. Stained cells were analyzed by LSR-II (BD Biosciences) and using FlowJo (version 3.6; Tree Star) software.

**RNA isolation and quantitative PCR analysis**

Total RNA from the colon tissues or LP lymphocytes was isolated using RNeasy kit (Qiagen), and cDNA was synthesized by reverse transcription. The mRNA levels of indicated mouse genes were determined by quantitative PCR analysis using the SYBR Green/ROX qPCR Master Mix (Thermo Scientific). All values were normalized to the housekeeping gene *actb* mRNA, and relative expressions were calculated by the ∆Δ cycle threshold method. Fold induction of genes was compared with the gene expression levels of uninfected mice.

**Statistical analysis**

Differences were tested using the Student *t* test. The *p* values are shown, and a *p* value <0.05 was considered statistically significant.

**Results**

**p38α in T cells, not in macrophages or DCs, plays an essential role in protection against *C. rodentium* infection**

We previously reported that p38α in IECs promotes T cell recruitment to provide protection against *C. rodentium* infection (24). To test the role of p38α in immune cells against *C. rodentium* infection, we generated macrophage-specific (p38α<sup>A<sub>D</sub>M</sup>) (17), DC-specific (p38α<sup>Adc</sup>), or T cell–specific (p38α<sup>aD</sup>) p38α-deficient mouse strains. Expression of p38α was defective in thymocytes, purified splenic T cells, and LP T cells, but was intact in other tissues and cells, including B cells and macrophages in p38α<sup>aD</sup> mice (Supplemental Fig. 1A, 1B). Flow cytometry analysis also confirmed the deletion of p38α in DCs of p38α<sup>Adc</sup> mice (Supplemental Fig. 1C). The ratio of CD4<sup>+</sup> and CD8<sup>+</sup> cells among CD3<sup>+</sup> T cells in the thymus, spleen, and LP was comparable between control (p38α<sup>Ad</sup>) and p38α<sup>aD</sup> mice (Supplemental Fig. 1D), indicating that deletion of p38α does not affect the development of T cells. Also, p38α-deficient T cells did not show any significant differences in TCR-mediated responses such as proliferation and IL-4 production compared with the control cells, whereas production of IFN-γ was significantly reduced in p38α-deficient T cells (Supplemental Fig. 2). This observation is cons-

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sistent with other reports using inhibitors or kinase-dead knock-in mutant mice (21, 28).

To test the role of p38α in immune cells against _C. rodentium_ infection, p38α<sup>A/B</sup>, p38α<sup>ΔMAC</sup>, p38α<sup>ΔDC</sup>, or p38α<sup>ΔT</sup> mice were orally inoculated with _C. rodentium_. Colon tissues were collected to measure _C. rodentium_ CFU after 1, 2, 3, or 4 wk postinfection. *p < 0.05, **p < 0.01. Error bars indicate SD. The results shown are representative of two to three experiments.

p38α regulates the production of inflammatory cytokines and the recruitment of T cells

We further investigated the mechanism of p38α-mediated T cell responses against _C. rodentium_ infection. Because DCs are on the front line of host defense against the bacterial infection, we tested whether the activation of DCs was affected in p38α<sup>ΔT</sup> mice. DCs were obtained from p38α<sup>A/B</sup> and p38α<sup>ΔT</sup> mice after 1 wk of _C. rodentium_ infection. No significant differences in the expression of surface markers like CD80, CD86, or MHC II, and production of the inflammatory cytokine TNF-α were observed in CD11c<sup>+</sup> DCs in MLNs of p38α<sup>A/B</sup> and p38α<sup>ΔT</sup> mice (Fig. 3A). Also, activation of DCs in LP was comparable between p38α<sup>A/B</sup> and p38α<sup>ΔT</sup> mice (data not shown). These results indicated deletion of p38α in T cells in mucosal immunity.
p38α in T cells did not overtly affect the function of DCs during *C. rodentium* infection.

We next examined the function of T cells in *C. rodentium*-infected p38αfl/fl and p38αΔT mice. Because the expression of IFN-γ and IL-17 is critical for the mucosal immunity against *C. rodentium* infection (6, 11), the intracellular levels of IFN-γ and IL-17 in the intestine-associated LP T lymphocytes were examined by flow cytometry. Expression of IFN-γ was significantly reduced in CD4+ T cells of p38αΔT mice compared with p38αfl/fl mice. However, abrogation of p38α in T cells did not affect the expression of IL-17, indicating that p38α regulates Th1 responses in bacterial infection (Fig. 3B). We further analyzed the expression of proinflammatory cytokines in LP lymphocytes in *C. rodentium*-infected p38αfl/fl and p38αΔT mice by quantitative PCR method. Consistent with the flow cytometry results, induction of IFN-γ was reduced in T cells from p38αΔT mice, whereas that of IL-17 was similar between p38αfl/fl and p38αΔT mice (Fig. 3C). Inflammatory cytokines such as IL-2, IL-12, and IL-22 are important components of T cell–mediated host defense against the enteric bacterial infections. Expression levels of IL-2, IL-12, and IL-22 were significantly reduced, whereas IL-10 level was not affected in p38αΔT mice, indicating that p38α regulated the production of inflammatory cytokines by T cells that are known to provide host protection against *C. rodentium* infection. Because the infiltration of inflammatory cells was reduced in the colon tissues of p38αΔT mice (Fig. 2A, Supplemental Fig. 3), the recruitment of immune cells into the colonic mucosa was further tested. Flow cytometry analysis of CD4+ T cells in isolated LP lymphocytes showed that recruitment of CD4+ T cells into the colonic mucosa in p38αΔT mice was decreased (Fig. 3D), which indicated that p38α in T cells also affected the infiltration of CD4+ T cells to the site of *C. rodentium* infection. These results suggested that p38α in T cells regulated the activation and infiltration of T cells to protect the host from infection by A/E bacterial pathogens.

**IEC function was affected by reduced T cell activation in *C. rodentium*-infected p38αΔT mice**

One of the functions of IECs is to maintain a protective barrier against luminal pathogens. The tight junctions between epithelial cells are known to play an important role in protecting against the translocation and escape of the enteric bacteria from the intestinal lumen (29). We tested the integrity of epithelial cells in p38αfl/fl and p38αΔT mice by measuring *C. rodentium* CFUs in liver tissues following infection (30). Bacterial counts in liver tissues were comparable between p38αfl/fl and p38αΔT mice after 1 or 2 wk of infection (Fig. 4A), suggesting that IEC barrier function was not affected by reduced activation of T cells in p38αΔT mice.

Next, we tested whether reduced T cell activation resulted in changes of mucosal defense function in p38αΔT mice by comparing the expression levels of proinflammatory cytokines, chemokines, and antimicrobial peptides in colon tissues. Ex vivo production of cytokines was measured by incubating colon tissue fragments from *C. rodentium*-infected mice, and we found that the levels of IFN-γ, IL-22, and IL-6 were lower in the colon of p38αΔT mice compared with control mice, whereas IL-17 and TNF levels did not differ (Fig. 4B, 4C). Expression of proinflammatory cytokines, chemokines, and antimicrobial peptides from colonic tissues of *C. rodentium*-infected p38αfl/fl and p38αΔT mice was further examined by quantitative PCR analysis (Fig. 4D). The mRNA levels of inflammatory cytokines in the colonic tissues were similar; IFN-γ, IL-22, and IL-6 levels were lower in the colon of p38αΔT mice, whereas IL-17 and TNF levels were comparable. We previously reported that p38α in IECs promotes the expression of chemokines such as CXCL10 and CCL25 that recruit T cells (24). In the colon tissue of p38αΔT mice, expression of inflammatory cytokines, chemokines, and antimicrobial peptides in colon tissues was compared between p38αfl/fl and p38αΔT mice by measuring the levels of IFN-γ, IL-22, and IL-6 from the colon tissues. Ex vivo production of cytokines was measured by incubating colon tissue fragments from *C. rodentium*-infected mice, and we found that the levels of IFN-γ, IL-22, and IL-6 were lower in the colon of p38αΔT mice compared with control mice, whereas IL-17 and TNF levels did not differ (Fig. 4B, 4C). Expression of proinflammatory cytokines, chemokines, and antimicrobial peptides from colonic tissues of *C. rodentium*-infected p38αfl/fl and p38αΔT mice was further examined by quantitative PCR analysis (Fig. 4D). The mRNA levels of inflammatory cytokines in the colonic tissues were similar; IFN-γ, IL-22, and IL-6 levels were lower in the colon of p38αΔT mice, whereas IL-17 and TNF levels were comparable. We previously reported that p38α in IECs promotes the expression of chemokines such as CXCL10 and CCL25 that recruit T cells (24). In the colon tissue of p38αΔT mice, expression...
of CXCL10 and CCL25 was significantly reduced, whereas CXCL2 levels were comparable (Fig. 4D). The expression of intestinal antimicrobial peptides did not differ between C. rodentium–infected p38αΔT and p38αfl/fl mice, except that expression of β-defensin 1 (Defb1) was significantly reduced in p38αΔT mice, indicating that Defb1 expression was regulated by inflammatory cytokines produced by T cells (Fig. 4D).

Induction of chemokine expression by IECs contributes to host defense against C. rodentium infection (4), and expression of chemokines in the intestinal mucosa regulates the recruitment of effector lymphocytes to the intestine (31, 32). Therefore, we examined the expression of cytokines and chemokines by IECs of C. rodentium–infected p38αfl/fl and p38αΔT mice. Similar to the colon tissues, expression level of IFN-γ and IL-22 was significantly lower in the IECs of p38αΔT mice. Also, expression of CXCL10 was significantly reduced in IECs of p38αΔT mice (Fig. 4E), indicating that p38α-mediated T cell activation regulated the expression of inflammatory cytokines and chemokines in the IECs of C. rodentium–infected mice, which further recruited T cells to the site of bacterial infection. These results suggested that p38α-mediated T cell activation limited the A/E bacterial burden by promoting defense mechanisms within the intestinal mucosa.

Treatment of IFN-γ activates the host defense against C. rodentium infection in vivo

Given the results that reduced expression of inflammatory cytokines such as IFN-γ by p38α-deficient T cells resulted in the impaired host defense against C. rodentium infection, we tested the recovery/promotion of host defense by IFN-γ treatment in C. rodentium infection. First, we examined whether IFN-γ administration recovered the reduced host defense activity of p38αΔT mice. Bacterial counts in colon tissues were lower, and the development of transmissible colonic hyperplasia was less significant in IFN-γ–treated p38αΔT mice compared with the mice treated with PBS (Fig. 5A, and data not shown), indicating that IFN-γ recovered the reduced host defense against the enteric bacterial infection that was impaired by p38α deletion in T cells. We further evaluated whether administration of IFN-γ enhanced the immune response of C. rodentium–infected wild-type mice. The bacterial CFU was significantly lower in the colon tissues by IFN-γ administration (Fig. 5B). Also, treatment of IFN-γ in-
increased the recruitment of inflammatory cells and ameliorated the colonic tissue damage (Fig. 5C, 5D). Recruitment of T cells to the site of bacterial infection and the expression of some essential genes for host defense were significantly upregulated in the C. rodentium–infected wild-type mice treated with IFN-γ (Fig. 5E, 5F), suggesting that administration of IFN-γ activated the mucosal immune response against C. rodentium infection.

In this study, we showed that p38α was essential for the T cell–mediated immune response against C. rodentium infection. Although p38α has been previously reported to play an important role in innate immune responses against many different types of microbial infections, we found that activation of p38α did not limit C. rodentium infection in macrophages and DCs. In line with the role of p38α in IECs regulating the recruitment of T cells, our current study suggests that p38α regulates T cell– and IEC-mediated host defense against A/E pathogen infection.

**Discussion**

NF-κB and p38 MAPK signaling pathways are critical in the development of host defense against pathogenic enteric bacterial infections. The NF-κB pathway is essential for maintaining immune homeostasis in IECs, as abrogation of NF-κB signaling within IECs dramatically impaired mucosal immune responses, dysregulated IEC integrity, and led to the subsequent failure to clear bacterial pathogen burdens (12, 33, 34). Using IEC-specific p38α-deficient mice, we previously showed that p38α in IECs plays a protective role in host defense against C. rodentium infection by recruiting T cells to the site of infection, whereas the immune functions were not affected (24). In this study, we demonstrated that mice lacking p38α in T cells, but not in macrophages or DCs, failed to clear C. rodentium infection, indicating that p38α regulates the adaptive immunity to limit the degree of A/E pathogen infection. In TLR-mediated innate immune responses, p38α regulates the activation of inflammatory signaling pathways (17). TLR2 is required in maintaining mucosal integrity, and MyD88-mediated signaling pathway is essential for a protective innate immune response by neutrophils in C. rodentium infection (4, 7). However, TLR4 deficiency showed a delayed spread and colonization of C. rodentium, indicating that TLR4-mediated responses against this A/E pathogen are not host protective despite that C. rodentium is a Gram-negative bacterium (35). Although p38α in IECs is important, p38α does not significantly affect the functions of macrophages and DCs against C. rodentium infection.

Abrogation of p38α in T cells resulted in impaired pathogen clearance from colon tissues due to the reduced production of inflammatory cytokines by T cells. Although the involvement of Th1 and Th17 T cell is a critical process in host defense against C. rodentium infection (3, 11, 36–38), Th1 responses such as IFN-γ production were regulated by p38α in T cells, whereas IL-17 expression was unaffected by the deletion of p38α. However, the role of p38α in Th17 differentiation is arguable. Whereas studies using pharmacological and dominant-negative approaches showed the role of p38α in T cell–intrinsic IL-17 expression (22, 39, 40), a recent report excluded the involvement of p38α signaling in Th17 differentiation (41). The underlying mechanism of the selective cytokine expression by p38α is unclear. Because activation of STAT1 is involved in IFN-γ production and STAT3 regulates the expression of IL-17 in TCR-mediated activation (42), the selective phosphorylation of STATs by p38α may regulate the cytokine production. We observed that phosphorylation of STAT1 was reduced in p38α-deficient T cells, whereas STAT3 activation was not affected (unpublished observations), suggesting that p38α may control the activation of STAT1, but not STAT3, in T cell–mediated host defense against C. rodentium infection.

Production of inflammatory cytokines and recruitment of T cells are important for host defense against C. rodentium infection (3, 6, 43, 44). C. rodentium infection induces the expression of inflammatory cytokines, chemokines, and antimicrobial peptides that participate in the host defense mediated by IECs against C. rodentium. In IECs, p38α regulates the expression of chemokines such as CXCL10 and CCL25 that are important for the recruitment of T cells to the site of infection to protect the host (24, 31). In the IECs of p38α−/− mice, the expression of several cytokines and chemokines was reduced, most notably CXCL10, IFN-γ, and IL-22. IFN-γ and IL-22 are known to induce the expression of CXCL10 and CCL25 in IECs, both of which are involved in the attraction of immune cells (45, 46). Importantly, similar cytokines and chemokines were downregulated in IECs of C. rodentium–infected p38α−/− and p38αΔIEC mice, indicating their significance in host defense against A/E pathogen infection for both cell types. Therefore, it is suggested that p38α plays a prominent role in both T cells and IECs against C. rodentium infection by promoting the production of proinflammatory cytokines and chemokines.

Production of IFN-γ was significantly reduced in C. rodentium–infected p38αΔIEC mice, and the frequency of T cells in the colonic mucosa was lower, indicating that p38α regulates not only cytokine production, but also recruitment of T cells to the site of infection by regulating the expression of chemokines by the IECs. Impaired bacterial clearance was recovered by IFN-γ administration in C. rodentium–infected p38αΔIEC mice, indicating the role of IFN-γ in mucosal immunity against the enteric bacterial infection. Also, administration of IFN-γ increased the expression of CXCL10 to enhance the host defense mechanism of C. rodentium–infected wild-type mice by recruiting T cells to the site of bacterial infection. Expression of IFN-γ and Defb1 was also increased, indicating that IFN-γ activated the mucosal immune response to protect a host from the enteric bacterial infection. Our data have suggested that a strategy that activates the host mucosal immunity can be used as a treatment of A/E pathogen infection. Treatment of the A/E pathogen infection such as E. coli O157:H7 is limited to the replacement of fluids and electrolytes to prevent dehydration because antibiotics may increase the chance of developing hemolytic uremic syndrome, a potentially fatal complication caused by Shiga toxin–mediated kidney failure (47). Therefore, activation of host mucosal immune response such as targeting the p38α signaling can be used as a potential future method for the treatment of A/E infections.

**Disclosures**

The authors have no financial conflicts of interest.

**References**


