Mast Cells Limit Systemic Bacterial Dissemination but Not Colitis in Response to *Citrobacter rodentium*

Olivia L. Wei, Ashley Hilliard, Daniel Kalman, and Melanie Sherman*

Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia

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Enteropathogenic *Escherichia coli* and enterohemorrhagic *E. coli* cause an inflammatory colitis in human patients characterized by neutrophil infiltration, proinflammatory cytokine expression, and crypt hyperplasia. *Citrobacter rodentium* causes a similar colitis in mice and serves as a model for enteropathogenic *E. coli* infection in humans. *C. rodentium* induces systemic T-cell-dependent antibody production that facilitates clearance of the bacteria and protects the host from reinfection. The role of innate immune cells in infectious colitis, however, is less well understood. In this study, we have determined the role of mast cells in the inflammatory response and disease induced by *C. rodentium*. Mice deficient in mast cells exhibit more severe colonic histopathology and have a higher mortality rate following infection with *C. rodentium* than do wild-type animals. Despite unimpaired neutrophil recruitment and lymphocyte activation, mast cell-deficient mice have a disseminated infection evident in crucial organ systems that contributes to sepsis. Importantly, mast cells also have the capacity to directly kill *C. rodentium*. Together, these results suggest that mast cells protect the host from systemic infection by reducing the bacterial load and preventing dissemination of the bacterium from the colon.

Enteric pathogens such as enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) attach to and colonize the host gastrointestinal tract and cause weight loss, diarrhea, crypt hyperplasia, and transient colitis. EPEC is a major contaminant in food and water sources in developing countries and causes significant increases in morbidity and mortality, primarily among children. EHEC contaminates food and water sources in industrialized nations and causes both diarrhea and hemolytic-uremic syndrome. A related pathogen, *Citrobacter rodentium*, has only about 50% of its genes in common with EPEC and EHEC but nevertheless induces disease in rodents that is virtually identical to that caused by EPEC and EHEC in humans. *C. rodentium* infection in mice is widely used as an animal model of pathogenic *E. coli* infection in humans (7, 9, 10, 18–21, 34, 42, 43, 45–47).

EPEC, EHEC, and *C. rodentium* all induce a characteristic lesion in the intestine of the host that is characterized by intimate attachment to the host intestinal epithelial cells and effacement of microvilli. Such attaching and effacing (A/E) lesions result from translocation of virulence proteins from the bacterium into the host cell (13, 22, 23, 26). These factors induce formation of membranous protrusions, called pedestals, beneath the bacterium, and anchor the bacterium to the host cell (reviewed in reference 36). Pedestal formation is required for subsequent disease (31, 37). EPEC, EHEC, and *C. rodentium* all contain a 35-kb pathogenicity island called LEE (locus of enterocyte effacement) that encodes ~41 virulence factors that are essential for the formation of A/E lesions and for disease. The virulence factors from the LEE loci of EPEC, EHEC, and *C. rodentium* are interchangeable (11, 16, 34).

Several observations suggest that inflammatory colitis induced by A/E pathogens results primarily from a deleterious host response induced by innate immune cells but not lymphocytes. First, application of heat-killed *C. rodentium* to artificially permeabilized colons causes an inflammatory disease that is nearly identical to that seen upon infection with live bacteria (21). Second, colonic inflammation, but not clearance, can occur in response to *C. rodentium* in the absence of B and T cells (42, 47). Finally, large numbers of neutrophils accumulate in the infected colon epithelium, creating crypt abscesses (21). Together, these data suggest that A/E lesions induce permeability of the epithelial barrier and that innate immune cells within the crypts or lamina propria, including epithelial cells, fibroblasts, mast cells, macrophages, and dendritic cells, appear capable of inducing inflammation and other symptoms associated with infectious colitis. These data and other evidence suggest that clearance of the bacterium additionally requires an adaptive immune response. For example, RAG1−/− mice, B-cell-deficient mice, and wild-type mice depleted of CD4+ T cells all develop severe colitis and large bacterial loads and have higher mortality in response to *C. rodentium* infection (7, 42, 47).

In contrast to the wealth of information on the requirement of the innate immune response for inflammation and the adaptive immune response for clearance and resolution of infection with *C. rodentium*, information on the precise mechanisms by which innate immune cells cause colonic inflammation is limited. In this paper, we focus on the role of mast cells in the inflammatory response to *C. rodentium*. Mast cells recognize and phagocytose bacteria, produce antimicrobial peptides (AMP), and secrete immunoregulatory cytokines (1, 2, 12, 27, 32, 49). In peritoneal infections, secretion of tumor necrosis factor alpha by mast cells causes recruitment of neutrophils,
which then destroy the bacteria (14, 30). Mast cells also regulate ion secretion, water balance, and epithelial permeability, which can induce the diarrhea associated with enteric infection. For example, with mast cell-deficient rodents, Purdue and coworkers demonstrated that mast cells facilitate translocation of bacteria across the intestinal barrier in response to food allergens and are required for inflammation and antigen-induced diarrhea (5, 8, 38). Mast cells are responsible for the inflammation induced by cholera toxin A in the small intestine (50) and are implicated in gastritis induced by Helicobacter pylori (35). Together, these results suggest that mast cells participate in the initiation of colitis and inflammation in the intestinal tract.

Here we set out to investigate the role of mast cells in the inflammatory response to C. rodentium infection in mice that lack mast cells (W/Wv). Surprisingly, C. rodentium infection of W/Wv mice resulted in more severe inflammation in the colon and increased production of proinflammatory cytokines in the absence of mast cells. W/Wv mice also had increased mortality as a result of bacteremia and tissue damage. Thus, these data suggest that rather than mediating the inflammatory responses, mast cells play a crucial protective role in the clearance of an enteric pathogenic bacterial infection by preventing bacterial dissemination to extracolonic tissues and sepsis.

**MATERIALS AND METHODS**

**Experimental animals and cell isolation.** WBB6F1-kitW/Wv (W/Wv) (24) female mice (4 to 8 weeks old), their female congenic WBB6F1-kit+/- littermates (wild-type), and female TCRβ-/- mice (33) were obtained from The Jackson Laboratory. Animal care was provided in accordance with protocols approved by the Institutional Animal Care and Use Committee of Emory University. To obtain bone marrow-derived mast cells (BMMC), bone marrow was harvested from both femurs of 6- to 8-week-old wild-type mice and cultured in complete RPMI medium (10% fetal bovine serum, 50 U of penicillin-streptomycin per ml, 50 μM 2-β-mercaptoethanol, 1 mM sodium pyruvate, 1x nonessential amino acids [Collgro; Fisher]) supplemented with 1 ng of interleukin-3 per ml and 20 ng of stem cell factor (SCF) per ml (R&D Systems, Minneapolis, Minn.). BMMC were used after a minimum of 4 weeks in culture at >95% purity, as determined by flow cytometric analysis and toluidine blue staining. For reconstitution, BMMC (5 x 10^4) in 200 μl of phosphate-buffered saline (PBS) were injected intravenously into W/Wv mice. Reconstituted mice were housed for 8 weeks following injection prior to infection with C. rodentium. Age-matched uninfected W/Wv and wild-type littermates were infected in the same experiment as controls. T cells were isolated from the spleens of wild-type and W/Wv mice with T-cell enrichment columns (R&D Systems) in accordance with the manufacturer’s directions. T cells (5 x 10^6) in 200 μl of PBS) were infected intravenously into TCRβ-/- mice. Reconstituted mice were infected 48 h after reconstitution. Concanaevalin A (ConA)-elicited polymorphonuclear neutrophils (PMN) were obtained by a single intraperitoneal injection of ConA (200 μg in 200 μl of PBS; Sigma). Six hours later, peritoneal cells were collected, washed three times, and used in killing assays. These cells were 80 to 90% PMN as identified by Giemsa stain (LabChem, Pittsburgh, Pa.). To obtain CD3+ T cells, spleens from wild-type mice were homogenized, red blood cells were hypotonically lysed, and the remaining cells were washed three times before application to T-cell enrichment columns in accordance with the manufacturer’s (R&D Systems) directions.

**Infection.** C. rodentium (ATCC 51116) bacteria were prepared by overnight culturing at 37°C in Luria broth (Becton Dickinson) without shaking. Cultures contained approximately 2.5 x 10^8 bacteria. The dosage was confirmed by retrospective plating on MacConkey agar; C. rodentium forms small pink colonies with white rims on this medium. Survival of infected mice and changes in body weight were monitored twice daily. Mice that lost more than 20% of their original weight were euthanized. For histology, colons, kidneys, and livers were removed at indicated times postinfection, fixed in 10% formalin, and embedded in paraffin. Sections (5 μm) were cut and stained with hematoxylin and eosin (H&E) or Giemsa stain. Crypt heights were measured by micrometry with a wide-field Zeiss microscope and Slidebook software (Intelligent Imaging Innovations). Well-oriented crypts were used for measurements, and data points represent three measurements of five colons per group. For colonization of tissues, small pieces (1 cm, approximately 0.2 g) of colon, liver, spleen, kidney, and lung were weighed and then homogenized at low speed with a TissuePrep (Fisher) in 1 ml of PBS as previously described (7, 11, 18, 41, 46, 47). Blood was collected daily after infection by ocular sinus puncture daily after infection. Serial dilutions were plated on MacConkey agar plates. C. rodentium colonies were recognized as pink with a white rim on MacConkey agar as previously described (46). Random colonies were confirmed as C. rodentium by PCR with TIR-specific primers (forward primer GCCGCGATTTCGCTTTAGGAAACTTCTTGTTGTAATAAAT and reverse primer GCGCCCCGGGGTAGACGCGACTCCCGGTGTTGT). Colonies were counted after 20 h of incubation at 37°C to determine the number of CFU per gram of tissue or per milliliter of blood. As expected, we found that blood and tissues from uninfected mice were not colonized by C. rodentium.

**ELISA for C. rodentium-specific antibody.** For antibody isotype analysis, anti-body titers in sera were determined by enzyme-linked immunosorbent assay (ELISA). Plates were coated with 100 μl of 10 μg of C. rodentium lysate per ml in 0.1 M carbonate buffer and incubated overnight at 4°C. The plates were then blocked with blocking solution (0.2% bovine serum albumin–PBS–Tween 20) at 37°C for 1 h. Dilutions of sera starting at 1:10 to 1:120 were made with blocking solution, and 100 μl of each dilution was added to duplicate wells of coated plates. After incubation at room temperature for 4 h, the coated plates were washed with PBS-polyoxyethylene sorbitan monooctanoate (Tween 20; Sigma) and bound immunoglobulin (Ig) was detected with biotin-conjugated goat anti-mouse IgG1, IgG2a, and IgG2b at room temperature for 2 h. The plates were then washed with PBS-Tween 20, and 100 μl of a 1:1,000 dilution of streptavidin-horseradish peroxidase in blocking solution was added for 1 h at room temperature. After incubation, color was developed with tetramethylbenzidine (Perox; Promega), stopped with 0.18 N sulfuric acid, and measured by determination of optical density at 450 nm on a microplate reader (Biotek). The antibody titer was defined as the dilution required to reduce a positive signal to threefold above the background.

**Flow cytometry.** Cells were harvested from lymph nodes and spleens of infected W/Wv or wild-type mice and subsequently stained with fluorescein isothiocyanate-labeled rat anti-mouse CD44 or CD3, phycoerythrin-labeled anti-CD4 or -CD25, or allophycocyanin-labeled anti-CD8 monoclonal antibody in accordance with the manufacturer’s (BD Bioscience, San Jose, Calif.) instructions. As isotype controls, we used the same concentration of rat IgG1, IgG2a, and IgG2b. To confirm cell maturation and differentiation, BMMC were blocked with antibodies to the Fcy receptors CD16 and CD32. Cells were incubated with murine IgE and then stained with directly conjugated monoclonal antibody anti-murine IgE (Becton Dickinson and Co, Mountain View, Calif.) or anti-murine IgG1, IgG2a, and IgG2b. Cell analysis was performed with a FACScalibur flow cytometer (Becton Dickinson & Co., Mountain View, Calif.) equipped with Cell Quest software.

**Killing assay.** C. rodentium bacteria were cultured overnight in Luria broth and serially diluted in PBS. Bacteria (2.5 x 10^8 CFU) were added to 2.5 x 10^6 BBMC, PMN, or T cells in 300 μl of RPMI medium without antibiotics. For killing by supernatant, mast cells were plated at a concentration of 5 x 10^5/ml and cultured for 24 h. The supernatant was then harvested, and 100 μl of mast cell supernatant was used for the killing assay. After 1, 2, or 3 h, the bacterium-cell culture was diluted 1:10 in water for 10 min to lyse the cells (39) and serial dilutions were plated on MacConkey agar. Cultures were set up in triplicate, and the results reported are representative of at least three independent experiments.

**MPO activity assay.** Colon tissue samples (0.2 to 0.3 g) were homogenized in ice-cold potassium phosphate buffer (50 mM K2HPO4, 50 mM KH2PO4, pH 6.0) containing hexadecyltrimethylammonium bromide (0.5% [wt/vol]; Sigma). The homogenate was then subjected to three freeze-thaw cycles, followed by sonication on ice for 10 s at power 5 before centrifugation at 14,000 rpm (Forma Scientific centrifuge model no. 120) for 15 min at 4°C. Aliquots of each supernatant or MPO (myeloperoxidase) standard (14 μg; Sigma) were added to 200 μl of substrate (0.167 mg of o-dianisidine per ml, 0.0005% H2O2 in potassium phosphate buffer), and the A460 was measured with a plate reader (Biotek). Protein levels were measured by the bichinchoninic acid protein assay (Bio-Rad). MPO activity is expressed as units per milligram of protein. One unit of enzyme activity is defined as the amount that consumes 1 μmol of H2O2/min.

**Statistical analysis.** All data are presented as mean values (± the standard error of the mean) unless otherwise designated. Comparisons between groups were made with a two-tailed Student t test, and statistical significance was defined for all tests as a P value of less than 0.05.
RESULTS

Mast cells contribute to clearance of *C. rodentium*. To determine the role of mast cells in infectious colitis, we used a well-characterized mouse strain, WBB6F1-Kit<sup>W</sup>Kit<sup>W</sup> (W/W<sup>v</sup>) (24, 25). Signaling from c-kit, the receptor for SCF, is defective in W/W<sup>v</sup> mice. Because SCF signaling is required for mast cell development, W/W<sup>v</sup> mice lack >99% of mast cells (24), and our experiments showed no detectable mast cells in colon tissue as assessed by Giemsa staining (e.g., see Fig. 2C). Five-week-old wild-type mice showed little outward sign of disease after oral infection with *C. rodentium*. In contrast, age-matched W/W<sup>v</sup> littermates had a ruffled appearance and a hunched posture and lost 10 to 20% of their original body weight. By day 7 postinfection, approximately 90% of W/W<sup>v</sup> mice succumbed, compared to 0% of control wild-type littermates (Fig. 1A). Thus, mice lacking mast cells display greater morbidity and mortality after infection with *C. rodentium*.

A hallmark feature of *C. rodentium* infection is colonic hyperplasia measured as an increase in crypt length. Such increases in crypt length are typically evident at 7 days postinfection and maximal after 10 days. To determine whether mast cells contribute to colonic hyperplasia, we measured crypt lengths in wild-type and W/W<sup>v</sup> mice left uninfected or infected with *C. rodentium*. Macroscopic thickening of the colon was observed in all infected mice (Fig. 1B to E), and crypt lengths increased to the same extent in wild-type and mast cell-deficient animals (Fig. 1F). Thus, although the absence of mast cells contributes to increased morbidity and mortality, it does not affect colonic hyperplasia.

To determine whether the greater morbidity and mortality of W/W<sup>v</sup> mice are due to the absence of mast cells, we reconstituted 5-week-old W/W<sup>v</sup> mice with cultured BMMC from wild-type mice. A second control W/W<sup>v</sup> group was left unreconstituted. After 4 weeks, the age-matched control W/W<sup>v</sup> group displayed very mild symptoms upon infection with *C. rodentium*, similar to those observed among age-matched wild-type littermates (Fig. 2A). Thus, the survival of W/W<sup>v</sup> mice showed some dependence on age, consistent with previous reports that older wild-type mice have less severe disease (28). Wild-type mice, reconstituted W/W<sup>v</sup> mice, and surviving W/W<sup>v</sup> mice were sacrificed on day 28, and the bacterial loads in their colons were quantitated by plating colonic homogenate on MacConkey agar plates (see Materials and Methods). *C. rodentium* was not evident in the colons of any mice, indicating that all had cleared the infection by this time (data not shown). These results suggest that the presence of mast cells decreases the morbidity and mortality associated with *C. rodentium* infection. Moreover, our observation that the W/W<sup>v</sup> mice that survive the infection can clear the bacteria suggests that mast cells are not required for an adaptive immune response or clearance of the infection (see also below).

*C. rodentium* spreads from the colon in mice lacking mast cells. To measure bacterial loads in blood and in various tissues following infection, we cultured the bacteria present in blood or in homogenates of liver, lung, kidney, and spleen tissues of
infected mice on MacConkey agar plates. After 7 days of infection, W/Wv mice had approximately 10-fold more bacteria in their colons than did reconstituted W/Wv mice, and C. rodentium was detectable in the blood, livers, lungs, kidneys, and spleens of W/Wv mice but not in the blood or tissues of reconstituted W/Wv mice (Fig. 3A and B). Consistent with the large bacterial load in the blood and peripheral organs of W/Wv mice, histological analysis revealed tissue necrosis and infiltration of neutrophils and T cells in focal abscesses in both the kidneys and livers of these mice (Fig. 3C). Together, large bacterial loads and histopathology are strong indicators of sepsis. No infiltration or signs of disease were evident in the kidneys or livers of wild-type mice or those of reconstituted W/Wv mice. These observations suggest that the presence of mast cells prevents dissemination of C. rodentium from the colon to the blood and tissues, and sepsis.

**Neutrophil recruitment to the infected colon is independent of mast cells.** C. rodentium induces a localized inflammatory response in the colon, characterized by infiltration of immune cells such as neutrophils and lymphocytes (21). W/Wv mice have been shown to be more susceptible to peritoneal infections caused by cecal ligation and puncture because of the absence of mast cell-derived tumor necrosis factor alpha. In these studies, neutrophils were not recruited to the peritoneum, which in turn resulted in greater bacterial loads and septic shock in the mast cell-deficient mice (14, 30). To investigate whether neutrophils are recruited to the colon in W/Wv mice infected with C. rodentium, we quantitated MPO levels in infected colon tissue from W/Wv and wild-type mice. In contrast to previous studies on peritoneal infections, MPO activity in the colons of W/Wv mice was two- to threefold greater than in wild-type mice, indicating that neutrophil recruitment to the colon is not impaired in the absence of mast cells (Fig. 4). In support of these results, we observed large numbers of neutrophils in the lamina propria of infected wild-type and W/Wv mice (Fig. 1D and E). To our knowledge, these data are the first to demonstrate a protective role for mast cells during a bacterial infection that is independent of neutrophil chemotaxis. T-cell activation and recruitment to the draining lymph nodes were also not affected by the absence of mast cells (see supplemental figure at http://kalmanlab.com/supplementary_figure.pdf). C. rodentium-specific antibodies are induced by day 14 postinfection in both groups of mice, and IgG1 and IgG2b isotypes are dominant in both strains with no significant differences in levels (Fig. 5). Together, these results suggest that mast cells are not required for neutrophil recruitment to the colon or the activation of an adaptive immune response to C. rodentium.

**Secreted factors derived from mast cells kill C. rodentium in vitro.** Our observation that the bacterial loads in W/Wv mice were greater than those in wild-type animals raised the possibility that mast cells directly reduce the bacterial load. Mast cells are known to bind to and phagocytose bacteria (reviewed in reference 15) and can also produce AMP (12). To test whether mast cells directly kill C. rodentium, live bacteria were
mixed with cultured mast cells (BMMC) and bacterial survival was quantitated after 0, 1, and 3 h of incubation. Surprisingly, mast cells are more potent than ConA-elicited PMN in their bactericidal ability (Fig. 6B), while T cells do not kill bacteria as expected. Consistent with the ability of mast cells to produce antibacterial peptides, supernatant from cultured mast cells also kills *C. rodentium* (Fig. 6A). Because mast cells are numerous in perivascular tissue, these results raise the possibility that mast cells may protect the host by secreting antibacterial factors around blood vessels and thereby reducing the number of *C. rodentium* bacteria spreading to extracolonic tissues.

**DISCUSSION**

*A/E* pathogens, including EPEC, EHEC, and *C. rodentium*, cause disease characterized by intestinal inflammation and diarrhea. Previous reports suggest that the inflammatory response occurs following a breach of the intestinal barrier and is mediated by innate and adaptive immune mechanisms (20). Whereas the adaptive responses to *A/E* pathogens have been well characterized, innate immune responses have not. We provide evidence here that rather than contributing to the inflammatory response, mast cells protect the host from bacteremia and sepsis. This protective function of mast cells is novel; previous reports have suggested that mast cells initiate or exacerbate tissue damage because of their capacity to release proinflammatory mediators, for example, during multiple sclerosis, inflammatory arthritis, and atopic dermatitis (reviewed in reference 44).

Control of bacterial dissemination following *C. rodentium* infection in the colon has to date only been associated with adaptive immune responses. Increased mortality and greater bacterial loads in the peritoneal cavity are evident in CD4- and RAG-1-deficient mice infected with *C. rodentium* (7, 42, 47). Likewise, infection of *β226/−* mice, which have an intact T-cell compartment but lack B cells, causes severe colitis and results in a greater pathogen burden in colonic and extracolonic tissues (7). Thus, protection from a disseminated infection and

**FIG. 3.** Mast cells help to prevent dissemination of *C. rodentium* in vivo and protect mice from organ failure caused by sepsis. (A) *C. rodentium* escapes from the site of infection to the blood in mast cell-deficient mice. Blood was collected from W/Wv mice 6 days after infection, and 50 μl was plated onto MacConkey agar plates and incubated for 20 h at 37°C before the colonies were counted. (B) *C. rodentium* bacteria were detected in the livers, lungs, and kidneys of W/Wv mice but were absent in both wild-type and reconstituted W/Wv mice. Various tissues of mast cell-deficient mice (W/Wv), wild-type littermates, and BMMC-reconstituted W/Wv mice were harvested aseptically 7 days after infection (n = 6 to 11 mice in each group), and *C. rodentium* CFU were counted as described in Materials and Methods. *, P < 0.05 compared with wild-type animals. (C) H&E-stained sections from wild-type and W/Wv mice showing that, 7 days after *C. rodentium* infection, significant amounts of cells in the livers and kidneys of W/Wv undergo apoptosis (arrows), which ultimately contributes to death.

**FIG. 4.** Neutrophil recruitment is independent of the presence of mast cells. MPO assay was used to examine neutrophil recruitment after infection of wild-type (WT) and W/Wv mice. Mast cell-deficient mice have two- to threefold more neutrophils in the colon than do wild-type mice (n = 6 to 11 mice in each group). *, P < 0.001 compared to wild-type mice.
clearance appear to require both T and B cells. It is interesting that the protective T- and B-cell response to \( C. \) rodentium is systemic, not localized to the colon. In this regard, Bry and Brenner have demonstrated that \( \alpha_2 \) integrin \(-/-\) mice, which are deficient in homing of lymphocytes to the intestinal mucosa and have impaired intestinal lymphoid responses, can still resolve a \( C. \) rodentium infection (7).

While an adaptive response appears necessary to control and clear an infection caused by \( C. \) rodentium, it does not appear to be sufficient. First, adaptive responses take up to 2 weeks to fully develop. Thus, innate immune cells may promote protection early in the infection or restrict the infection to the colon. Second, innate immune cells appear to offer protection against death associated with disseminated infection in mice lacking lymphocyte responses. For example, mortality rates of RAG \(^{1-/-} \) mice in response to \( C. \) rodentium infection vary from 5\% (42) to 100\% (7). Some of the variance in these studies may be attributable to factors such as age, commensal flora, and diet, all of which may contribute to the virulence of the bacteria (28, 48). Nevertheless, even taking these factors into account, a significant proportion of mice can survive a disseminated infection without T- or B-cell responses, suggesting that the innate immune system also participates in host protection. Our data provide evidence that mast cells may mediate such a protective response by directly killing the bacteria (Fig. 6).

The high mortality rate in mast cell-deficient mice after infection with \( C. \) rodentium appears to be due to bacteremia and sepsis, which are due not to an increase in the bacterial load in the colon but rather to an inability to limit the bacteria to this site. Bacteria are detected in the blood, livers, and lungs of infected mice that lack mast cells (Fig. 3). The serum of these mice also has increased levels of alanine transaminase, which is associated with liver damage (data not shown), and liver and kidney sections showed evidence of necrosis and infiltration of inflammatory cells, symptoms commonly associated with sepsis. Notably, similar phenotypes are evident in mice lacking B- or T-cell responses (7, 29, 42, 47), suggesting...
that wild-type mice likely require several immune mechanisms to protect the host and clear the infection.

Our results indicate that mast cells do not participate in the recruitment of other innate immune effector cells to the infected colon, nor do they appear to have a protective role at this site. Accordingly, the large intestine has very few mast cells compared with the stomach, jejunum, and small intestine (17). Although mast cells have been shown to attract neutrophils to the peritoneum following infection with *E. coli* (14, 30), we find that neutrophil infiltration in *C. rodentium*-infected W/W<sup>+</sup> mice is unimpaired, even enhanced, which suggests that other cell types can provide signals required for PMN recruitment to the colon. In accordance with this idea, EPEC has been found to induce epithelial cells to produce interleukin-8 and stimulate neutrophil migration in vitro (40).

Our data do suggest several means by which mast cells protect the host systemically from bacteremia and sepsis following infection. First, mast cells may facilitate tissue repair of epithelial monolayers and thereby prevent further bacterial entry. A critical component of the disease process induced by A/E pathogens is a breach in epithelial barrier integrity. Genes within the LEE pathogenicity island, a locus present in all A/E pathogens, appear to mediate such breaches, and a barrier breach is required for the development of inflammation (20). Mechanisms within epithelial cells may participate in repairing the monolayer; in vitro evidence indicates that primary epithelial cells grow to contact inhibition following wounding of the monolayer. To date, little information is available on intrinsic mechanisms of wound healing after A/E bacterial infection. However, mast cells have been implicated in tissue repair of heart epithelial tissue following ischemia in vivo (3) and have been shown to be a rich source of epithelial and fibroblast growth factors (4) that stimulate epithelial cell growth. Thus, it is possible that mast cells in the intestinal mucosa repair breaks between cells in the colonic epithelium, thereby restoring barrier integrity and preventing continued bacterial access. To test this hypothesis, experiments are currently under way to determine whether mast cells home to barrier breach sites during infection.

A second mechanism by which mast cells may protect the host from bacteremia and sepsis is to kill bacteria directly. We present evidence showing that mast cells can kill *C. rodentium* by secreting a factor or factors with antimicrobial activity. Di Nardo et al. have shown that AMP are present in mast cells and are secreted upon stimulation with various bacteria (12). Up to 45 separate AMP-encoding genes are contained in the mouse genome (6), and it has been demonstrated that the expression of two of these AMP, mBD-1 and mBD-3, was upregulated in colon tissue following infection with *C. rodentium* (43). We have preliminary data showing that mast cells produce mBD-3 and that mBD-3, but not mBD-1, is sufficient to kill *C. rodentium* (O.L.W., unpublished observations). Whether the bactericidal activity of mast cells is due to mBD-3, or another AMP, is under investigation. Killing of bacteria in the colon by AMP may not be mediated exclusively by mast cells; epithelial cells also express AMP. Moreover, the colon may not be the only site where killing occurs. As noted above, systemic immune responses are required for clearance. Mast cells are present in large numbers in the fibroblast layers surrounding blood vessels, and mast cells at these sites may prevent access of spreading bacteria to organ systems. Testing of this model is currently under way with mutant strains of *C. rodentium* resistant to killing by AMP.

In summary, secretion of AMP by several cell types, including mast cells, may be required to control the bacterial load systemically and thereby restrict the infection to the colon. In the absence of mast cells, unrestricted bacteriological growth appears to permit the bacteria to reach extraluminal sites and cause tissue damage. Mast cells may also repair barrier breaches in the intestinal epithelium and prevent continued access of bacteria to submucosal space. Failure of mast cells to perform these functions may result in sepsis and death following enteric infection.

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**REFERENCES**


