Commentary

The Role of Mast Cells in Bacterial Enteritis

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Considering that the intestinal lumen is home to greater than 400 commensal bacterial species, it is curious that the mucosal immune system can detect the presence of pathogens among benign flora. The rapid and potent response of the innate immune cells ensures that virulent organisms are contained to the intestine, although this reaction can also damage surrounding tissue if not kept in balance. The work by Feng et al¹ in this issue of *The American Journal of Pathology* demonstrates that mast cells are involved in the inflammatory reaction to intestinal pathogens and that mast cell products released during degranulation may be the cause of intestinal damage due to infection.

Several innate immune cell types reside in the intestinal lamina propria, including mast cells, dendritic cells, and macrophages. Within the intestine, mast cells are found in the mucosal and submucosal layer and can release a variety of bioactive proteins that can directly affect ion secretion (leading to diarrhea) and epithelial barrier function (leading to bacterial translocation). Although it is well documented that mast cell activation contributes to secretory diarrhea and colitis, it is still unclear how mast cells are triggered by microbial products to cause pathology. The observations presented here by Feng et al¹ demonstrate that Toll-like receptor (TLR)2 on mast cells is responsible for diarrhea caused by *Staphylococcus aureus*. This work is a culmination of past studies demonstrating that mast cell products affect intestinal physiology and that mast cells are an important part of the innate immune response to bacterial infection.

What Is the Evidence That Mast Cells Influence Gastrointestinal Function?

Mast cells were first observed to be increased in tissue biopsies from patients with inflammatory bowel disease.² The increased number of mast cells also associates with evidence of mast cell degranulation and the extent of degranulation correlates with the degree of inflammation. It is worth noting that mast cells appear to be grouped

specifically along the line of demarcation separating healthy tissue from inflamed tissue in ulcerative colitis patients³ and also with strictures in Crohn's disease.⁴ A second observation is the reactivity of pANCA with mast cells. The generation of pANCA antibodies is found in 68 to 80% of ulcerative colitis patients and is thought to be either a preclinical marker for disease or a susceptibility factor.⁵ Importantly, pANCA reacts to a cytoplasmic factor found in mucosal mast cells located in the colon.⁶ These observations suggest that mast cells are associated with intestinal inflammation and can be a target for autoimmune antibody development.

However, conclusive evidence comes from studies using mast cell-deficient animals. *Ws/Ws* rats, which have a small deletion in the *c-kit* gene and therefore do not develop mast cells, are resistant to experimentally induced colitis with dextran sodium sulfate.⁷ Mast cell-deficient mice are resistant to enteritis caused by *Clostridium difficile* toxin,⁸ and mice that have been deleted of mast cells with *c-kit* antibody are resistant to diarrhea caused by oral allergens.⁹ In this issue of the *AJP*, Feng et al¹ demonstrate that mast cell-deficient mice are resistant to peptidoglycan-induced diarrhea and that the mast cell stabilizer ketotifen can prevent gastrointestinal pathology caused by *S. aureus*. Reconstitution of the mast cell-deficient mice with mast cells recovers the inflammatory response. Thus, these experiments suggest that mast cells or their products contribute to intestinal inflammation and therefore are possible therapeutic targets for patients with acute or chronic diarrhea.

How Do Mast Cells Cause Inflammation?

Mast cells are unique in that they store preformed inflammatory mediators in their granules, poised for immediate release on activation. Like other immune cells, mast cells can also provide sustained release of these products as a result of *de novo* synthesis. Granules can also be re-

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generated for later cycles of degranulation from the same mast cell, demonstrating the potency of these cells and their potential for serious effects on surrounding tissue.¹⁰

Mast cell granules contain a variety of bioactive proteins that cause inflammation. The neutrophil chemoattractants leukotriene B₄, interleukin 8 (keratinocyte-derived chemokine in rodents), macrophage inflammatory protein-2, and tumor necrosis factor- α (TNF- α) are all prestored in mast cells and can be released within seconds of cell activation (see review¹¹). In addition, histamine and serotonin will induce vascular permeability, which allows influx of fluid, immune cells, and proteins to the damaged tissue.¹² A placebo-controlled, double-blind study using human volunteers that were orally challenged with 75 mg of pure histamine demonstrated that histamine induces gastrointestinal symptoms within 3 to 24 hours after ingestion.¹³ Interestingly, common foods such as cheese, tuna, and tomatoes can contain up to 500 mg/kg histamine and may contribute to the development of food sensitivities.

In a rat model using cholera toxin to induce secretory diarrhea, the intestinal fluid released after toxin injection contains the cytokines interleukin 1 β and TNF- α .¹⁴ These cytokines are made in large quantities by mast cells; in fact, the majority of TNF- α -producing cells in intestinal lamina propria are mast cells.¹⁵ The release of cytokines into the intestinal lumen can induce the inflammatory cascade resulting in colitis.

How Do Mast Cells Detect Bacteria?

Studies performed in the 1970s demonstrated that mast cells can interact directly with pathogens through complement receptor 3 and Fc γ R after opsonization.¹⁰ Other studies by Malaviya et al¹⁶ demonstrated that mast cells recognize the fimbrial lectin FimH on *E. coli* through CD48. The direct binding of bacteria by mast cells leads to phagocytosis and killing of the bacteria, demonstrating that mast cells can act as a first line of defense against bacterial infections (see review¹⁰).

Mast cells also express TLRs and can react to microbial products, as shown here by Feng et al.¹ TLRs comprise a family of cell-surface receptors that bind to various microbial molecular patterns and stimulate proinflammatory cytokine production (see review¹⁷). Human mast cells derived from cord blood were demonstrated to engulf and destroy a variety of bacterial strains while secreting considerable amounts of TNF- α .¹⁸ Mast cells express TLR2, TLR4, TLR6, and TLR8,^{19,20} allowing detection of peptidoglycan from gram-positive bacteria and lipopolysaccharide from gram-negative bacteria among other bacterial products.

Mast cell-deficient mice are acutely sensitive to peritonitis because of a lack of neutrophil recruitment by mast cell TNF- α .²¹ Reconstitution of mast cell-deficient mice with wild-type mast cells rescues the mice and allows clearance of the bacteria, but reconstitution with TLR4-defective mast cells correlates with poor neutrophil recruitment and higher mortality.¹⁹ These studies demon-

strate that TLR signaling in mast cells is crucial for innate immune protection from bacterial infection.

Interestingly, Supajatura et al²² demonstrated that TLR2 and TLR4 signaling in murine mast cells show quantitative and qualitative differences in cytokine production. Importantly, TLR2- but not TLR4-dependent mast cell activation results in degranulation. Moreover, intradermal injection of peptidoglycan induces vasodilation and inflammation of the skin via TLR2 signaling in mast cells.²² These previous observations support the work of Yang et al,¹ demonstrating that peptidoglycan activation of mast cells in the intestinal tract can also cause inflammation and vasodilation.

Are Mast Cells a Target for Therapy?

Ketotifen, a potent mast cell stabilizer, has been used to control asthma allergy but has also been shown to effectively protect against enteritis. In a rat model of clostridium toxin disease, ketotifen prevents intestinal damage.²³ Ketotifen is also effective against cholera toxin in a rat ileum model.¹⁴ Importantly, ketotifen leads to improvement or remission in human patients suffering from inflammatory bowel disease.^{24,25} The Feng et al study¹ presented in this issue of the *AJP* broadens the effects of ketotifen to gram-positive bacterial infection in mice. Moreover, a combination of histamine H1 antagonist and 5-hydroxytryptamine 3 antagonist also blocked peptidoglycan-induced diarrhea. Together, these studies suggest that mast cells contribute to intestinal disease because of their sensitivity to bacterial products and ability to release potent inflammatory mediators, suggesting a potential therapeutic target for bacterial enteritis in combination with antibiotics.

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