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Pathogenesis, Immunology and Diagnosis of Porcine Proliferative Enteropathy

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Introduction

Proliferative enteropathy (ileitis) is an infectious, intestinal hyperplastic disease characterized by thickening of the intestinal mucosa due to enterocyte proliferation. The cause of proliferative enteropathy is the obligate intracellular bacterium *Lawsonia intracellularis*, which preferentially grows within the cytoplasm of intestinal epithelial cells. Proliferative enteropathy has been reported in several animal species but has been best described in pigs and hamsters. Various names have been used, including proliferative enteritis, porcine intestinal adenomatosis, proliferative hemorrhagic enteropathy, ileitis, wet-tail disease, and intestinal adenomatous hyperplasia. The two major clinical forms of proliferative enteropathy in pigs are acute hemorrhagic diarrhea (sudden death of replacement animals and pigs close to market age, known as proliferative hemorrhagic enteropathy) and chronic mild diarrhea and reduced performance in growing pigs, known as porcine intestinal adenomatosis.

Pathogenesis

Cell proliferation, the primary feature of proliferative enteropathy, has not been reproduced in vitro. As a result, most studies on the pathogenesis of *L. intracellularis* have been conducted in vivo. Comprehensive studies of lesion development and evolution have been conducted in hamsters and pigs. Morphological studies of early lesions in experimentally-infected animals indicate that enterocyte hyperplasia is directly preceded by the presence of the intracellular organism. In vivo, the onset of hyperplasia associated with proliferative enteropathy follows an increase in numbers of intracellular *L. intracellularis* in enterocytes. Likewise, resolution of lesions is closely related to disappearance of the intracellular organisms, indicating a correlation between the two events. The means by which *L. intracellularis* produces hyperplasia is unknown. No other cytopathologic effects on infected enterocytes are seen in vivo or in vitro. Inflammation is a factor only in later-stage lesions and is not a characteristic of the primary lesion.

Progression of infection

Studies describing the development and evolution of proliferative lesions have been conducted. Conventional or gnotobiotic pigs colonized by nonpathogenic enteric flora and inoculated with pure cultures of *L. intracellularis* have consistently developed clinical signs and macroscopic lesions of proliferative enteropathy. Three weeks following oral inoculation with *L. intracellularis*, intracellular organisms, as well as enterocyte proliferation, were identified in intestinal sections. Early lesions of proliferative enteropathy were studied in vivo in pigs and hamsters by electron microscopy. *L. intracellularis* in the crypt lumen associates with the cell membrane and enters the immature epithelial cell via an entry vacuole. The vacuole breaks down and bacteria multiply freely within the cytoplasm. These infected epithelial cells continue to undergo mitosis, transmitting the organisms to daughter cells. Eventually, the bacteria are released from cytoplasmic extrusions on the epithelial cells on top of the villi or between crypts. Infection spreads to the entire ileum, distal jejunum, caecum, and colon. M (Microfold) cells may be involved in the pathogenesis of *L. intracellularis* infection as proliferative enteropathy lesions in early stages of infection appear in the Peyer’s patch area of the intestine. Studies have examined the progression of gross and histological lesions through the course of experimentally-produced proliferative enteropathy in pigs. Macroscopic lesions were first detected 11 days after infection. Histological lesions characterized by enterocyte hyperplasia and reduction of goblet cells were first observed 11 days after inoculation. *L. intracellularis* antigen was first detected in the intestine by immunohistochemistry 5 days after inoculation. Positive staining, but no gross or histological lesions, was detected on day 29. Pigs euthanized on day 35 post-inoculation had no lesions and were negative by immunohistochemical staining. Conversely, one challenged pig was PCR positive on day 35 showing that, despite the non-detection of the bacterium by immunohistochemistry in examined intestinal fragments; there were still sites of infection in the gastrointestinal tract.
No *L. intracellularis* antigen was detected in any organ besides intestine, lymph node and tonsil. It appears that *L. intracellularis* infection is limited to enterocytes and the bacterial antigen found in the lamina propria and mesenteric lymph nodes may simply be carried there by macrophages. Infection of enterocytes in the large intestine and rectum occurs later in the course of the disease and, consequently, the infection can be detected later in these locations. It appears that the small intestine is infected first and the bacteria shed from those sites infect lower levels of the intestine. Tonsil does not appear to play a role in pathogenesis, but tonsillar crypt cells may have *L. intracellularis* antigen in their cytoplasm.

After the successful growth and maintenance of *L. intracellularis* in vitro, it was possible to understand some mechanisms involved in the bacterial entrance into eukaryotic cells and the evolution of the infection in cell cultures. Infection of cell cultures in vitro show many of the features of the disease in vivo. The entry process is dependent on cell activity and independent of bacterial viability. Close association between *L. intracellularis* and cultured enterocyte cell surface was observed 10 minutes after infection. Multiplication of the bacteria by binary fission free in the cytoplasm was observed from two to six days after cell culture infection. Drugs that inhibit cell growth also inhibit multiplication of *L. intracellularis*, indicating that cell division is required for bacterial multiplication. Six days after infection, highly infected eukaryotic cells have balloon-like, cytoplasmic protrusions, replete with bacteria that are then released from the cell.

Virulence factors

The virulence factors of *L. intracellularis* have not yet been fully elucidated. *Lawsonia*’s major pathogenic mechanism is infection of and induction of hyperplasia in enterocytes. Generally, no significant inflammatory response is reported and the infection remains localized in enterocytes. Attachment and entry of this bacterium into immature epithelial cells occurs at the apical surface. Specific adhesins or receptors for *L. intracellularis* have not been characterized, but attachment and entry appear to require specific bacterium-host cell interaction.

Most studies on the pathogenesis of *L. intracellularis* have been conducted in vivo since the cellular proliferation that is characteristic of proliferative enteropathy has not been reproduced in vitro. The mechanism by which *L. intracellularis* induces cell proliferation is unknown. In vivo, proliferating enterocytes show poor major histocompatibility complex class II expression. This loss of antigen-presenting function may provide an immunological safe environment for *L. intracellularis* to grow. Temporary reduction of apoptosis induced by *L. intracellularis* infection might be one of the mechanisms involved in enterocyte proliferation. Absence of the bacteria has been associated with resumption of apoptotic events in the intestinal mucosa and an increase of normal epithelial cells in recovering lesions. Another study has shown an increase of apoptosis in hyperplastic crypts and villi of pigs with naturally occurring proliferative enteropathy. Hyperplastic crypts in the ileum, presumably highly infected by *L. intracellularis*, had significantly more apoptotic cells, detected by caspase-3 immunohistochemistry stain (*p*<0.0002), than normal crypts. It appears that cell proliferation, a characteristic feature of proliferative enteropathy, is not caused by reduction of apoptosis.

Very little is known about the genetic basis for the virulence, pathogenesis, or physiology of *L. intracellularis*. Further, the molecular mechanisms for infection and virulence and the epidemiology of this organism in pigs and other species remain undetermined. A whole genome sequencing approach identified several sequences in *L. intracellularis* of interest from a virulence standpoint. Sequences homologous to genes encoding proteins involved in flagellar biosynthesis and assembly have been identified. Identification of a flagellum in *L. intracellularis* isolates, coupled with the sequences that correspond to regions of genes involved in flagellar assembly, provides a means of developing specific reagents to delineate its role in virulence and infectivity. Sequence analysis also identified a homolog to a membrane-bound Yop (Yersinia outer protein) and a homolog of LvrV, confirming that *L. intracellularis* is likely to contain a type III secretion system.

Immune response

Information has become available about the humoral and cellular immune responses in pigs naturally or experimentally infected with *L. intracellularis*. IgG could be first detected two weeks after challenge of 5-week-old pigs with pure culture of *L. intracellularis*. Antibody levels (IgG) peaked around the end of the third week and then tended to drop.
Convalescent pigs do have immunity to re-inoculation. Animals challenged a second time, after cessation of fecal shedding, did not shed *L. intracellularis* and had no clinical signs. Bacteria in the second challenge may have been inactivated before entry and colonization of mucosal cells. The cell-mediated immune response is an important feature of infections caused by intracellular organisms. Descriptive immunocyto logical studies of intestinal tissue sections of pigs affected by both clinical forms of proliferative enteropathy reveal a mild infiltration of cytotoxic T cells, macrophages and B lymphocytes carrying MHC class II structure at the beginning of the cell-mediated immune response. Mucosal local humoral immunity, in the form of secreted IgA, is also a relevant defense mechanism against enteropathogenic microorganisms. Immunohistochemical studies of intestinal sections of pigs naturally affected by proliferative enteropa thy demonstrated a large accumulation of IgA in the apical cytoplasm of proliferating enterocytes. Interferon gamma is produced by PBMC’s following specific stimulation and IgA is detected in intestinal lavages of challenged pigs. The onset and duration of systemic cell-mediated and humoral immune responses and fecal shedding in pigs experimentally infected with pure culture of *L. intracellularis* was accessed. Humoral and cell-mediated immune responses were detected two weeks after exposure in pigs challenged with the pathogenic isolate. Humoral and cell-mediated immune responses were still detectable in some pigs 13 weeks after exposure. Fecal shedding was initially detected one week and lasted intermittently, 12 weeks post exposure in challenged pigs. Similarly, interferon gamma played a role in limiting intracellular infection and increased cellular proliferation in experimentally infected mice. Thus, animals exposed to a pathogenic pure culture isolate of *L. intracellularis* demonstrated long-term shedding of and specific immune responses to the organism.

**Diagnosis**

Clinically, proliferative enteropathy is difficult to diagnose because the signs are non-specific or lacking. One hallmark of the disease is dramatic weight variation among pigs of the same age. Chronic disease is characterized by poor growth, uneven weight gain and a delay to market. Overall poor performance, gauntness or soft-to-watery stools may occur. Conventionally, proliferative enteropathy has been diagnosed postmortem based on gross lesions seen at necropsy and microscopic tissue examination. Severe lesions are easily seen, however the more common moderate to mild lesions may be hard to detect. Gross lesions vary depending on the clinical manifestation of the disease and may appear as hemorrhagic or chronic. Histopathological examination of tissues can confirm a diagnosis. Though diagnosis can be made by demonstrating the presence of proliferative enterocytes on routine H&E staining, evaluating proliferation may be subjective; only cases with typical severe enterocyte proliferation can be reliably diagnosed. Staining of histological sections using a silver stain reveals numerous intracellular organisms with a characteristic curved shape, usually in the apical cytoplasm of the crypt epithelial cells. However, this method is not specific for *L. intracellularis* and cannot always detect the organism in necrotic debris or autolyzed tissue. More specific identification of *L. intracellularis* can be achieved by immunohistochemistry staining of fixed tissues, which is more sensitive than the silver stain because it shows organisms within macrophages of the lamina propria during recovery from disease.

Several techniques have been described for detecting *L. intracellularis* in live pigs. These include detection of *L. intracellularis* in feces by PCR or indirect antibody staining and serological assays for *L. intracellularis* antibodies.

**Conclusion**

*L. intracellularis* is a unique bacterium, which causes an unusual pathology in infected animals. Limited knowledge of the pathogenesis of *L. intracellularis* suggests that this organism has adopted mechanisms of survival and pathogenesis unique from those utilized by other bacterial pathogens.
Selected References


