

Mycobacterium avium Subspecies *Paratuberculosis* Triggers Intestinal Pathophysiologic Changes in Beige/Scid Mice

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We investigated whether infection of beige/scid mice with *Mycobacterium avium* subspecies *paratuberculosis* can induce intestinal pathophysiologic changes. Six-week-old beige/scid mice were inoculated intraperitoneally with *M. paratuberculosis*, then were killed 32 weeks after inoculation when the small intestine was evaluated for physiologic and morphologic abnormalities. All infected mice developed clinical disease. The lamina propria of the intestine from infected mice was mildly infiltrated with mononuclear cells containing acid-fast bacteria, and had significantly increased villus width. In vitro physiologic studies in Ussing chambers indicated that *M. paratuberculosis* infection caused significant abnormalities in intestinal transport parameters. Baseline short circuit current and potential difference were abnormally high in tissues from infected, compared with control mice, indicative of increased ion secretion. Baseline conductance was significantly decreased in infected mice, suggesting that intestinal tissue from infected mice was less permeable to ions. The change in short circuit current following transmural electrical and glucose stimulation was significantly reduced in intestines from infected mice, suggesting that inflamed intestine had neural and/or epithelial cell damage. We conclude that infection of beige/scid mice with *M. paratuberculosis* triggers significant intestinal pathophysiologic changes consistent with chronic inflammation. These functional abnormalities may contribute to the pathogenesis of the wasting syndrome seen in bovids with paratuberculosis. This animal model provides evidence that T cell-independent mechanisms are sufficient to cause mucosal pathophysiologic changes and inflammation in response to a specific pathogen, and may be of relevance to inflammatory bowel disease in humans.

Paratuberculosis (Johne's disease), a chronic granulomatous enteritis of ruminants, is caused by infection with *Mycobacterium avium* subspecies *paratuberculosis*. The clinical disease is characterized by long incubation period, progressive weight loss, diarrhea, and death (1, 2). Although it is principally an infection of ruminants, naturally acquired paratuberculosis in nonhuman primates has been reported (3). Additionally, *M. paratuberculosis* has been implicated as a cause of Crohn's disease, an inflammatory bowel disease (IBD) of humans, and is raising concerns that *M. paratuberculosis* could be a potential zoonosis (4, 5).

Although paratuberculosis has been recognized for about a century, its pathogenesis is still not well understood. The weight loss observed in animals with clinical paratuberculosis is thought to be due to several factors including: an intestinal pathophysiologic mechanism that leads to a protein-losing enteropathy (6), immune mediators released during immune responses to *M. paratuberculosis* (1, 7, 8), and reduced food intake, presumably due to reduced appetite (9). However, the exact mechanisms responsible for the weight loss have not been defined. Lack of a suitable laboratory animal model has been identified as a major hindrance to research of paratuberculosis (10, 11).

We previously reported the potential of severe combined im-

mune-deficient beige mice infected with *M. paratuberculosis* for research of paratuberculosis. Doubly mutant beige/beige.scid/scid (beige/scid) mice lack functional T and B lymphocytes, and have reduced natural killer (NK) cell activity (12-14). Animal models deficient in various immune components have been used extensively in studies of IBD of humans, the pathogenesis of which is thought to bear some similarities to that of Johne's disease. In this regard, rodents lacking interleukin (IL-) 2, IL-10, T-cell receptor α/β , interferon- γ , or IL-4, and even scid mice are known to develop intestinal inflammation due to dysregulated immune responses to normal gastrointestinal tract flora or to specific bacterial agents (15-20). Research using immune-deficient animals has allowed identification of how and why particular immunologic defects lead to mucosal inflammation. In this regard, evidence has accumulated indicating a requirement for lymphocytes (and their mediators) and the presence of bacteria in the lamina propria to provoke immune responses responsible for intestinal inflammation (21).

In our previous report (22), beige/scid mice infected with *M. paratuberculosis* developed progressive weight loss, and *M. paratuberculosis* localized in the lamina propria of small intestine in association with mononuclear cells. There did not appear to be any major alterations in the morphology of the intestinal epithelial barrier; however, we did not evaluate the physiologic status of the intestinal tissue. Since intestinal inflammation is often associated with alterations in physiologic functions, such as increased ion secretion, we hypothesized that infection of beige/scid mice with *M. paratuberculosis* could trigger significant in-

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testinal pathophysiologic changes, probably before morphologic changes became apparent. Thus, the main objective of the study reported here was to evaluate intestinal pathophysiologic changes caused by infection of beige/scid mice with *M. paratuberculosis*. Identifying these pathophysiologic mechanisms may contribute to our understanding of the pathogenesis of the chronic weight loss seen in animals with clinical paratuberculosis. Additionally, since beige/scid mice lack lymphocytes, results of this study may provide useful information regarding T cell-independent mechanisms in the pathogenesis of mucosal inflammation.

Materials and Methods

Animal inoculation. The care and use of animals was as recommended in the *Guide to the Care and Use of Experimental Animals* issued by the Canadian Council on Animal Care, and was approved by the Animal Care Committee of the University of Guelph. B6;CB17-*Lyst^{bgJ}-Prkdc^{scid}* (beige/scid) mice were bred by Dr. Anne Croy (University of Guelph) from flora defined (by use of Modified Schaedler Cocktail) CB-17-*Prkdc^{scid}* males and C57BL/6J-*Lyst^{bgJ}* females. Males were obtained from Dr. R.A. Philips (Toronto, Ontario, Canada), whose stock was obtained from Dr. M. Bosma (Fox Chase Cancer Institute, Philadelphia, Pa.); females were purchased from Jackson Laboratory (Bar Harbor, Maine). Mice were housed in a pathogen-free environment in microisolator cages (LAB Products, Inc., Federalburg, Md.) in a Horsfall unit. Mice were fed gamma-irradiated mouse chow (Charles River Laboratories, St. Contant, Quebec, Canada) and autoclaved water, and were observed daily.

Twenty-four beige/scid mice, approximately 6 weeks old and of either sex were assigned to three test and one control group ($n = 6/\text{group}$). The inoculum was prepared as described (22). Briefly, a suspension containing *M. paratuberculosis* (approx. 10^5 colony-forming units [cfu]/ml) was serially diluted to contain either 10^3 , 10^2 , or 10 cfu of *M. paratuberculosis*. A sample from each of these dilutions was cultured on Herrold's egg yolk medium containing mycobactin J, results of which confirmed viability and concentration of each in the three inocula. Each mouse in the three test groups was inoculated intraperitoneally once with 10 , 10^2 , or 10^3 cfu of *M. paratuberculosis*. Control mice were inoculated with saline.

Sample collection, assessment of clinical disease, and pathologic examination. Mice were observed for any evidence of disease during the experimental period. At 32 weeks after inoculation, mice were visually inspected and assigned a subjective clinical score on a scale from 0 to 5. A score of 0 represented a clinically normal mouse, and a score of 5 indicated a very sick mouse; the score assigned to each mouse was based on the severity of clinical signs of disease described in this animal model (22), and included roughness of coat, poor body condition, and huddling in the cage. The mice were anesthetized with methofane (methoxyflurane, Pitman Moore, Mississauga, Ontario, Canada), and a surgical incision was made into the abdomen on the ventral midline. Approximately 20 cm of the small intestine, from the distal portion of the ileum proximad, was excised, immediately placed in Krebs's buffer, and cut into three lengths of 12 cm (for mucosal scrapings), 7 cm (for Ussing chamber study), and 1 cm (the most distal portion, for histologic examination). The 7-cm mid-section was immediately transferred to warm (37°C), oxygenated (95% O_2 ; 5% CO_2) Krebs solution containing 10 mM mannitol (pH 7.35).

Each carcass was then inspected for gross lesions. Sections of the liver, spleen, and small intestine were dissected out and fixed in buffered 10% formalin for histologic examination. The 1-cm sections of intestine were opened at the mesenteric border, placed in Carnoy's fixative, embedded, sectioned, and stained with hematoxylin and eosin (H&E). Sections were coded and examined in blinded manner by one researcher who was not aware of the experimental mouse groups. Villus length, and width and crypt length were measured under light microscopy in selected fields containing at least three consecutive well-oriented crypt-villus units with a uniform epithelial layer. At least 10 measurements per section were made to obtain an average value. Measurements were done using a calibrated micrometer eyepiece. Infiltration of the lamina propria and submucosa with inflammatory cells was examined by use of light microscopy.

Additionally, formalin-fixed organs (spleen, liver and small intestine) were trimmed and embedded in paraffin, then consecutive sections were stained by use of either H&E (for identification of lesions) or the Ziehl-Neelsen technique (for identification of acid-fast organisms) and were examined by use of light microscopy.

Physiologic study in Ussing chambers. Physiologic intestinal transport parameters were studied in Ussing chambers in vitro, using a described technique (23). Briefly, a 7-cm segment of intestinal tissue was opened longitudinally at the mesenteric border and cut into four equal lengths. Each piece was rinsed in the buffer solution to dislodge any ingesta and was immediately mounted onto an Ussing chamber (WPI Instruments, Narco scientific, Mississauga, Ontario, Canada), which included removable stimulating electrodes on opposite sides of the tissue. The mounted surface area of 0.6 cm^2 was exposed to Krebs's buffer (115 mM NaCl, 8 mM KCl, 1.25 mM CaCl_2 , 1.2 mM MgCl_2 , 2.0 mM KHP0_4 , pH 7.35 ± 0.02), which, on the serosal side contained 10 mM glucose (serosal buffer), and on the mucosal side, contained 10 mM mannitol (mucosal buffer). The tissue was short-circuited at zero potential, using an automatic voltage clamp (WPI Instruments) and the short-circuit current (I_{sc} in $\mu\text{A}/\text{cm}^2$) was recorded continuously on a chart recorder. Potential difference (PD, mV) was determined at 10-min intervals, and conductance (G), measured in millisiemens per square centimeter (mS/cm^2), was calculated as follows; $G = I_{sc}/\text{PD}/0.6 \text{ cm}^2$ (24). The mucosal and serosal buffer solutions were constantly aerated by use of 95% O_2 /5% CO_2 , and were maintained at 37°C .

Measurement of baseline parameters. After tissues were mounted on Ussing chambers, they were allowed to equilibrate for 20 min, until steady baseline I_{sc} was observed. The circuit was then opened to measure the PD, and conductance (G), in mS/cm^2 , was determined.

Measurement of tissue response to electrical and chemical stimulation. After a further 15 min, the tissues received transmural electrical stimulation by administration of electrical stimulating current pulses (10 Hz, 10 mA, 0.5 msec) for a total of 5 sec. across the tissue in a perpendicular direction (25). The electrical stimulation causes mucosal nerves to release neurotransmitters and the increase in short-circuit current (ΔI_{sc}) following stimulation was recorded as the maximal change in current within 15 min.

The intestinal segments were also exposed to glucose and histamine, and maximal ΔI_{sc} was recorded. The ΔI_{sc} in response to stimulation with $10^{-3}M$ histamine (Sigma Chemical Co. St. Louis, Mo.) added to the serosal side of the intestinal preparation (se-

rosal buffer), 10 mM glucose added to the mucosal side (mucosal buffer), and $10^{-6}M$ forskolin (Sigma Chemical Co.) simultaneously added to the serosal and mucosal buffers after 40 min was recorded. All these stimuli are known to induce consistent secretory responses (23, 26).

Measurement of enzymes. Intestine mucosal enzyme activity was determined as described (27). Briefly, mucosa was scraped from the 12-cm segment of intestine, using the edge of a microscopic slide. Mucosal homogenates were manually prepared in 2.5 mM EDTA (adjusted to pH 7.5 with TRIS) in a glass homogenizer (100 mg/ml), were quick-frozen in vials in a dry ice-acetone bath, and were stored at $-70^{\circ}C$ until assayed. Enzymatic activity was measured in the thawed homogenate and expressed as units of activity per gram of mucosal protein by use of described methods; sucrase and maltase (28), alkaline phosphatase (29), and thymidine kinase (30) were determined as modified by Kertzner and co-workers (31). Protein content was determined as described by Lowry and co-workers (32).

Statistical analysis. Data were analyzed by use of one-way analysis of variance (ANOVA), and the means were ranked, using Duncan's classification approach ($P < 0.05$).

Results

Clinical disease and pathologic changes. Two mice from each of the four groups died of unknown causes during the experimental period. Control mice did not develop any evidence of clinical disease. In contrast, mice injected with any of the three doses of *M. paratuberculosis* developed clinical disease, and the severity of the clinical disease was *M. paratuberculosis* dose dependent. Mean clinical scores after 32 weeks of infection were 1.5, 4.0, and 5.0 for groups inoculated with 10, 10^2 , and 10^3 cfu of *M. paratuberculosis*, respectively (mean clinical score for controls was 0).

The major gross lesions observed at necropsy were loss of abdominal fat, enlargement of the spleen, mottling and discoloration of the liver, muscular wasting, and thickening of the wall of the small intestine. The extent of these lesions corresponded with numbers of *M. paratuberculosis* inoculated into the mice. The lesions were most extensive in mice that were inoculated with 10^3 organisms, moderate in mice inoculated with 10^2 organisms, and slight or inapparent in mice inoculated with 10 organisms. Gross lesions were not seen in control mice inoculated with saline.

The effect of *M. paratuberculosis* infection on the morphology of small intestine was evaluated by use of microscopic examination of intestinal sections. Infection with 10^3 cfu of *M. paratuberculosis* reduced villus length, compared with that of controls, but this was not statistically significant (Table 1). Villus width was increased in intestinal sections from infected mice, and was significantly ($P < 0.05$) increased in mice inoculated with 10^3 organisms, compared with controls and with the other two infected groups. Infection with 10 and 10^2 organisms increased crypt length, compared with that in controls, and this increase was statistically significant in the group of mice inoculated with 10^2 organisms.

The lamina propria of the gastrointestinal tract from infected mice was mildly infiltrated with inflammatory cells containing acid-fast bacilli, but these histopathologic changes were not seen in control mice. Examination of H&E-stained sections did not reveal significant differences in the degree of inflammation among the infected groups. The liver and spleen lesions consisted of mononuclear cells containing numerous acid-fast bacteria, the extent of which correlated with the dose of organisms in the inoculum.

Table 1. Measurements in microns (mean \pm SD) of distal small intestine villus plus crypt (V + C) length, villus (V) length and width, and crypt (C) length of control mice and three groups of beige/scid mice 32 weeks after a single intraperitoneal injection of 10, 10^2 , or 10^3 cfu of *M. paratuberculosis*

Treatment group	V+C-length	V-length	V-width	C-length
Control	0.543 \pm 0.002 ^{ab}	0.425 \pm 0.04	0.065 \pm 0.006 ^b	0.117 \pm 0.019 ^b
10 cfu	0.523 \pm 0.053 ^{ab}	0.404 \pm 0.06	0.071 \pm 0.003 ^b	0.122 \pm 0.007 ^{ab}
10^2 cfu	0.614 \pm 0.47 ^a	0.462 \pm 0.02	0.068 \pm 0.007 ^b	0.151 \pm 0.027 ^a
10^3 cfu	0.479 \pm 0.031 ^b	0.374 \pm 0.04	0.096 \pm 0.007 ^a	0.106 \pm 0.009 ^b

^{ab}Different superscript letters on the same column denote significant ($P < 0.05$) difference. N = 4 for all groups. Length and width of villus and crypt depth were measured on the longest three to six consecutive villi, using a calibrated micrometer eyepiece on H&E-stained, well oriented, blinded sections.

Effect of *M. paratuberculosis* infection on intestinal enzymes. Sucrase activity was reduced in mucosal scrapings from all infected groups of mice, compared with that in scrapings from uninfected controls, and the difference was statistically significant in the group inoculated with 10^2 organisms. The activities of all other enzymes were not significantly different (data not shown).

Effect of *M. paratuberculosis* infection on intestinal physiology. The effect of *M. paratuberculosis* infection on intestinal physiology was evaluated by measuring electrical transport parameters in Ussing chambers in vitro. Baseline electrical parameters indicated that Isc and PD values were increased (indicative of increased ion secretion) in tissues from all infected mice, compared with values for uninfected controls, and this increase was statistically significant (Fig. 1A and 1B). Additionally, conductance (G) was significantly reduced in all the infected groups of mice, compared with that in controls (Fig. 1C).

When normal intestinal tissue from control mice was subjected to electrical stimulation (causes release of endogenous neurotransmitters), there was a transient large Δ Isc, indicative of a net secretion of ions (Fig. 2A). In contrast, when intestinal tissue from *M. paratuberculosis*-infected mice was subjected to electrical stimulation (TS), there was a significant reduction in Isc response, compared with that in controls (Fig. 2A). The reduction in short circuit current was similar in infected mice regardless of the dose of *M. paratuberculosis* inoculated.

Luminal exposure of intestinal tissue to glucose normally stimulates Na/glucose cotransport, reflected as a large Δ Isc. This Δ Isc, in response to glucose stimulation, was significantly reduced in intestinal tissue from the three groups of mice infected with *M. paratuberculosis* mice, compared with that in controls (Fig. 2B). This reduction in Δ Isc appeared to correlate with the number of organisms used for inoculation. Thus, mice inoculated with the highest number of organisms (10^3 cfu) had the smallest response, whereas the group of mice inoculated with the least number of organisms (10 cfu) had the largest response, but differences among the infected groups of mice were not statistically significant.

The response to histamine appeared reduced in the test, compared that in control mice, but the difference was not statistically significant (Fig. 2C). The Δ Isc in the test groups in response to forskolin was similar to that in controls.

Discussion

Results of this study clearly indicate that infection of beige/scid mice with *M. paratuberculosis* induced significant intestinal pathophysiological changes, as indicated by abnormalities in transport function-associated parameters. The observed intestinal pathophysiological changes occurred in association with mild

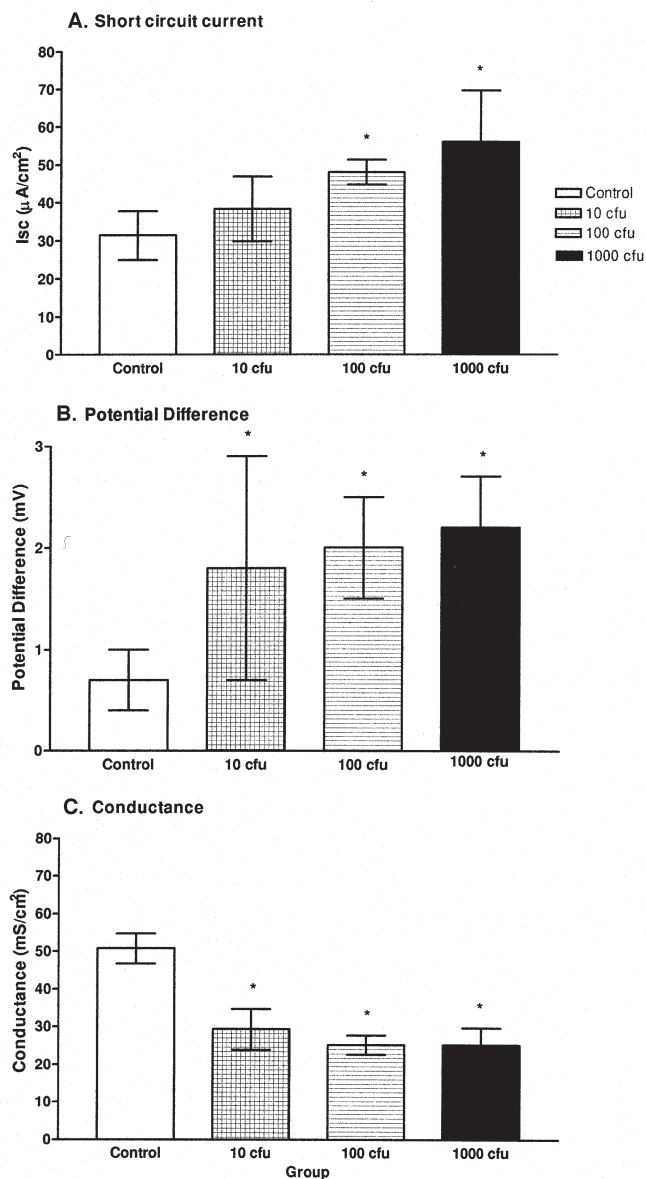


Figure 1. Graphs illustrating baseline (A) short circuit current, (B) potential difference, and (C) conductance in small intestinal segments from control and beige/scid mice infected with 10, 100, and 1,000 colony-forming units (cfu) of *Mycobacterium paratuberculosis*. (n = 4/group, with three to four replicate tissues from each animal; *P < 0.05, compared with controls by use of one-way analysis of variance [ANOVA]).

inflammatory responses in the lamina propria. Thus, significant pathophysiologic changes can develop before significant morphologic changes are evident.

Increased ion secretion and abnormalities of the intestinal barrier often are associated with intestinal inflammation. We used an approach similar to that used by other investigators to study pathophysiologic changes in models of intestinal inflammation (23, 33). It was documented that intestinal inflammation in *Nippostrongylus braziliensis*-infected rats caused net secretion of Na^+ and Cl^- , which consequently increased baseline Isc and PD values (23). This is in agreement with our study in which significantly high baseline Isc and PD values were observed in intestinal tissue from beige/scid mice infected with *M. paratu-*

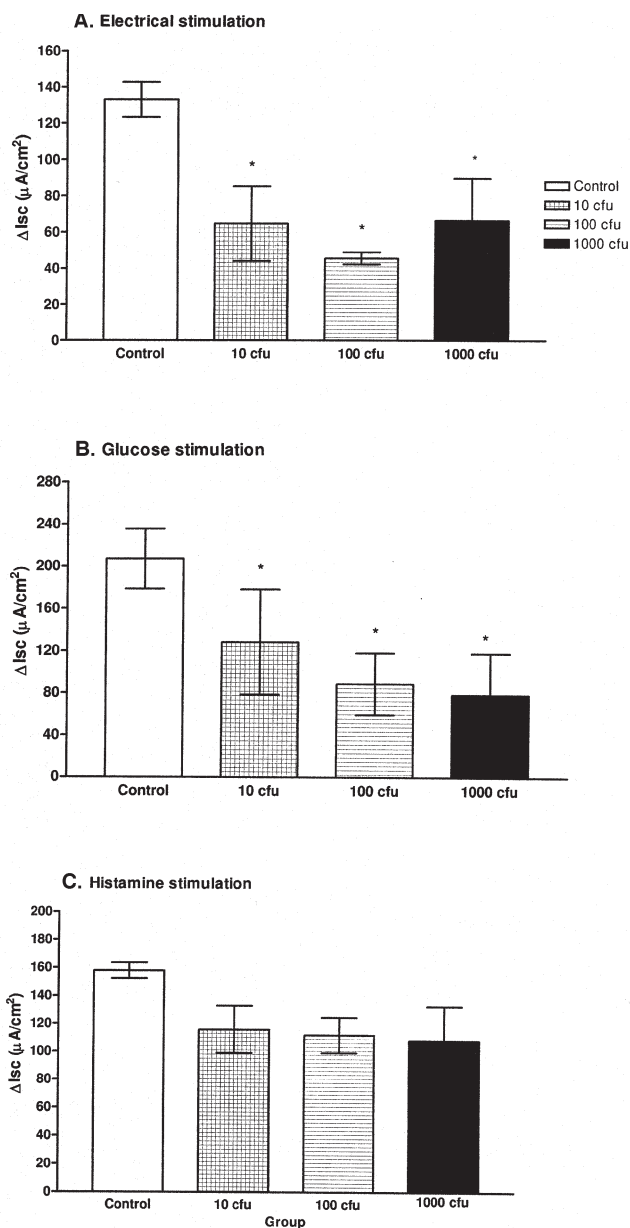


Figure 2. Graphs illustrating change in short circuit current (ΔIsc) in response to (A) transmural electrical stimulation, (B) exogenous glucose, and (C) exogenous histamine in small intestinal segments from control and beige/scid mice infected with 10, 100, or 1,000 cfu of *M. paratuberculosis*. (n = 4/group, with three to four replicate tissues from each animal; *P < 0.05, compared with controls by use of ANOVA).

berculosis. Conductance can often be used as an indicator of permeability of the intestinal barrier to ions. In our study, baseline G was decreased in all infected beige/scid mice, indicating that the inflamed intestine of these mice was less permeable to ions and consequently restricted transmural movement of ions. These observations from our study are indicative of intestinal pathophysiologic changes, and would be consistent with chronic inflammation of the small intestine of the beige/scid mouse caused by *M. paratuberculosis* infection.

The responses of the small intestine to various stimuli were reduced in beige/scid mice infected with *M. paratuberculosis*. Transmural electrical stimulation of the intestine in vitro usu-

ally would stimulate mucosal nerves to release endogenous neurotransmitters, which in turn would induce secretion of Cl^- to the luminal side of the intestinal epithelium (25), consequently increasing change in ΔIsc . In our study, it would seem that ΔIsc in response to transmural electrical stimulation was markedly reduced due to intestinal inflammation caused by infection with *M. paratuberculosis*. Our observation is in agreement with that of others, in which ΔIsc response to transmural electrical stimulation was reduced during intestinal inflammation, and the poor response was due to abnormality of mucosal nerves (23). In our study, however, it is unclear whether the reduced ΔIsc is due to a neuronal defect (inability to release neurotransmitters) or an epithelial abnormality (inability to respond to neurotransmitters), or both.

The ΔIsc in response to glucose stimulation of the intestinal mucosa was significantly decreased in infected mice in a manner related to the dose of *M. paratuberculosis* used. The transport of glucose is carrier mediated, and is associated with the absorption of Na^+ . Thus, a relative decrease in the Isc response to glucose stimulation would suggest a defect in the carrier system, which worsened with severity of the infection. This may have important implications in the pathogenesis of the weight loss seen in animals with paratuberculosis since other molecules of dietary significance, such as amino acids, also are absorbed by specific but different carriers. Malabsorption of amino acids is thought to be a contributing factor in the pathogenesis of the weight loss seen in bovines with paratuberculosis (6) and in mice (34).

The ΔIsc in response to addition of histamine was reduced in the intestines of infected mice, regardless of dose of *M. paratuberculosis*. Intestinal epithelial cells secrete Cl^- on exposure to histamine, which is reflected as a change in Isc . Histamine mediates its effects in intestinal tissue by occupying H_1 -receptors on enterocytes and neurons (35, 36) and H_2 -receptors in enteric nerves. Decreased response to histamine may reflect an epithelial or neuronal defect, or both. Responses to another ion secretion stimulator, forskolin, did not differ between control and infected mice. The stimulatory activity of forskolin is mediated directly via activation of cAMP, which is the major secondary messenger responsible for ion secretion. The fact that responses to forskolin remained unchanged is interesting and indicates that some of the pathways of induction of ion secretion are not affected by infection with *M. paratuberculosis*, whereas others, such as the glucose carrier system and histamine responses, are changed following infection.

Physiologic abnormalities observed in intestine of infected mice were similar among the infected groups of mice. Additionally, there was mild infiltration of the lamina propria with inflammatory cells, and the degree of infiltration did not differ significantly among the infected groups. Thus, significant functional abnormalities may occur early in the inflammatory process, before any significant morphologic changes are apparent.

Intestinal inflammation during paratuberculous enteritis is believed to be partly due to abnormal or excess immune responses against *M. paratuberculosis* (1, 37). Similarly, dysregulated mucosal immune responses in the intestine are believed to cause IBD in humans. Research with immune-deficient animal models of IBD indicate that T lymphocytes (principally CD4^+ T lymphocytes) play a pivotal role in the pathophysiologic changes and tissue damage associated with intestinal inflammation (16, 18, 21). In this regard, in vivo neutralization of the Th1 cytokine $\text{IFN-}\gamma$ resulted in signifi-

cant reduction of intestinal inflammation (38). Scid mice, and even scid/beige mice, which lack functional lymphocytes, are known to produce the cytokines implicated in intestinal inflammation, including $\text{IFN-}\gamma$ and tumor necrosis factor (TNF)- α (39-41). We speculate that, in our study, the presence of *M. paratuberculosis* in the lamina propria of beige/scid mice triggers recruitment of inflammatory cells, such as macrophages (and likely NK cells), to secrete cytokines, such as $\text{IFN-}\gamma$ and TNF- α , which have been implicated in intestinal inflammation. However, further experiments are required to confirm the specific cytokines that cause the observed pathophysiologic changes. Our observation that *M. paratuberculosis*-infected beige/scid mice (which lack functional T and B lymphocytes) developed intestinal pathophysiologic changes suggests that there are T cell-independent mechanisms that are sufficient to cause intestinal inflammation. Other investigators have reported that scid mice infected with *M. avium* have the capacity to recruit macrophages and form organized granulomas in the absence of functional T cells (41).

Although results of this study indicate that T cell-independent mechanisms are sufficient to cause intestinal pathophysiologic changes (and inflammation), T cells certainly contribute to the pathogenesis of severe intestinal pathophysiologic changes. Thymectomized rats (which lack functional T lymphocytes), had less severe injury to the intestinal mucosa, compared with that in conventional rats following parasitic infection (42). In separate experiments in our laboratory, we found that adoptive transfer of T and B lymphocytes from immune-competent BALB/c mice to beige/scid mice is associated with induction of interstitial pathophysiologic changes (unpublished observation). Thus, whereas T cells may not be essential to initiate intestinal inflammation, lymphocytes certainly appear to contribute substantially to the pathogenesis of severe intestinal inflammation.

In the study reported here, we documented that an infection was established in beige/scid mice following a single intraperitoneal inoculation of as few as 10 cfu of *M. paratuberculosis*. The lesions in the liver, spleen, and intestine of all infected mice were consistent with those of our previous report (22). Increasing the dose of organisms merely reduced time to establish the infection. The severity and extent of the clinical signs of disease and macroscopic lesions appeared to correlate with the numbers of *M. paratuberculosis* organisms inoculated. Infiltration of the lamina propria and submucosa with inflammatory cells may have contributed to the significant increase in villus width that was observed in the group that was infected with 10^3 cfu of *M. paratuberculosis*.

It was surprising that thymidine kinase activity (an indicator of cell proliferation) was not increased in the intestinal tissue of infected mice. Other than the sucrase activity in the group infected with 10^2 cfu, which was significantly reduced below that in controls, the activity of the other enzymes appeared normal. This would support the histologic impression that the epithelium of the distal portion of the small intestine had mild morphologic (structural) changes despite the presence of large numbers of *M. paratuberculosis* in the submucosa and the lamina propria. Often, acute intestinal inflammation is associated with a mucosal lesion that consists of villus atrophy and crypt hyperplasia (42). In such instances, the infective agent damages the epithelium, predominantly at the villus tips, removing an inhibiting factor that regulates proliferation in the crypts. In our study, the lack of morphologic changes to the intestinal epithelium may be due to the localization of *M. paratuberculosis* in the lamina propria

of the villus, with sparing of the enterocyte. Furthermore, *M. paratuberculosis* does not produce any known toxin.

Further work needs to be done to determine whether a link exists between these observed functional abnormalities of the distal portion of the small intestine and the cachexia that develops when beige/scid mice are chronically infected with *M. paratuberculosis*. This may provide avenues for future research into the specific causes of the cachexia associated with paratuberculosis in cattle.

Although intraperitoneal inoculation consistently induces intestinal lesions in our animal model, natural infection in animals is acquired principally through oral transmission. However, earlier studies using the oral inoculation route indicated that infection was established in only 50% of the beige/scid mice (22). This reflects the situation in the field in which only a fraction of animals exposed to *M. paratuberculosis* actually become infected. Oral administration of *M. paratuberculosis* would require a higher dose of organisms to initiate infection because of physiologic barriers, such as gastric acidity, intestinal/pancreatic enzymes, and clearing effects of peristalsis.

This beige/scid mouse model is important in that intestinal pathophysiologic changes (hence, inflammation) can be induced in the absence of T lymphocytes after chronic infection with a specific pathogen. This also is important in view of the fact that *M. paratuberculosis* causes chronic enteritis in animals and has been implicated in some cases of Crohn's disease (4).

In conclusion, chronic *M. paratuberculosis* infection in beige/scid mice caused significant intestinal pathophysiologic changes in association with mild inflammation in the lamina propria, and only minor alteration in tissue morphology. These results further indicate that significant pathophysiologic changes and inflammation can develop in the intestine in the absence of T cells.

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