Neutrophils Do Not Mediate the Pathophysiological Sequelae of Cryptosporidium parvum Infection in Neonatal Piglets

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Cryptosporidium parvum is a minimally invasive protozoal pathogen of intestinal epithelium that results in villus atrophy, mucosal lipid peroxidation, diarrhea, and diminished barrier function. Influx of neutrophils is a consistent feature of human and animal cryptosporidiosis, and yet their contribution to the pathological sequelae of infection has not been investigated. Accordingly, we used an established neonatal piglet model of C. parvum infection to examine the role of neutrophils in disease pathogenesis by inhibiting their recruitment and activation in vivo using a monoclonal anti-CD18 antibody. Infected piglets were treated daily with anti-CD18 or isotype control immunoglobulin G and euthanized at peak infection, at which time neutrophil infiltration, lipid peroxidation, severity of infection, and intestinal barrier function were quantified. C. parvum infection resulted in a significant increase in mucosal neutrophil myeloperoxidase activity that was prevented by treatment of piglets with anti-CD18 antibody. Neutrophil recruitment was dependent on mucosal superoxide formation (prevented by treatment of infected piglets with superoxide dismutase). Neutrophils did not contribute to peroxynitrite formation or peroxidative injury of C. parvum-infected mucosa and had no impact on the severity of epithelial injury, villus atrophy, or diarrhea. The presence of neutrophils in C. parvum-infected mucosa was associated with enhanced barrier function that could not be attributed to mucosal elaboration of prostaglandins or stimulation of their synthesis. These studies are the first to demonstrate that neutrophilic inflammation arising in response to infection by a noninvasive epithelial pathogen results in physiologic rather than pathological effects in vivo.

In this study, we demonstrate for the first time that neutrophils have minimal impact in mediating the pathological sequelae of C. parvum infection. Infection of neonatal piglets with C. parvum resulted in significant villus atrophy, diarrhea, mucosal lipid peroxidation, and recruitment of neutrophils into the lamina propria. Neutrophil recruitment was dependent on superoxide formation by the mucosa (inhibited by superoxide dismutase [SOD]) and blocked by treatment of piglets with anti-CD18 antibody. Neutrophil depletion did not ameliorate lipid peroxidation or peroxynitrite formation, suggesting that these cells are not a significant source of free radicals in C. parvum-infected mucosa. Further, mediators derived from or stimulated by neutrophils did not play a significant role in the genesis of diarrhea, as diarrheal severity, villus atrophy, and epithelial Cl− secretion were unaffected by neutrophil depletion. Finally, barrier function of C. parvum-infected mucosa was significantly worsened, rather than improved, by the absence of neutrophils. These observations suggest that in noninvasive infection of intestinal epithelium, influx of neutrophils promotes physiologic rather than pathological effects in vivo.

MATERIALS AND METHODS

Animals. Experimental animals were 1-day-old crossbred piglets obtained from the College of Agriculture. Piglets were placed into infected and control isolation units and fed a liquid diet by an automated delivery system. An inoculum of 10^9 C. parvum oocysts (Bunch Grass Farms, Deary, ID) was given to piglets by orogastric tube on day 3 of life. Control and infected piglets were studied on days 3 to 5 after inoculation, a time span shown previously to be inclusive of peak intestinal infection (3). Piglets were euthanized using sodium pentobarbital given intravenously (i.v.), and sections of ileum, beginning 5 cm...
above the ileocecal junction, were taken sequentially for histology, in vitro function testing, and assays. All infected piglets used in the study showed evidence of villus atrophy and organisms adherent to villus enterocytes, whereas control piglets showed normal villus architecture with no evidence of infection. All studies were approved by the Institutional Animal Care and Use Committee.

**Morphometric analyses.** Sections of ileum were fixed in formalin, paraffin embedded, sectioned at 5 μm, and stained with hematoxylin and eosin for examination by light microscopy. Thrice sections from each tissue were examined. Three to five well-oriented villi were selected by an examiner blinded to treatment category. Villi were considered well-oriented if the adjacent crypt lumen was patent to the level of the muscularis mucosa. Average villus height (from the crypt opening to the villus tip) and crypt depth were measured using an ocular micrometer, and the percentage of epithelialized villus surface was calculated from linear measurements of epithelialized versus denuded villus perimeter. The total number of villus epithelial cells and total number of intracellular parasites along the perimeter of each of the selected villi were counted.

**Measurement of lipid peroxidation.** Thiobarbituric acid-reactive substances were measured in homogenates of ileal mucosa on the basis of the formation of a colored adduct of malondialdehyde (MDA) with 2-thiobarbituric acid. An 800-μl aliquot of homogenate was added to a reagent solution containing 20% acetic acid (1.5 ml), 8.1% sodium dodecyl sulfate (200 μl), and 0.8% 2-thiobarbituric acid and 0.05% butylated hydroxytoluene (1.5 ml). The mixture was boiled for 1 h and then cooled. After cooling, the sample products were measured with 5 ml of n-butanol and pyridine (15:1). The absorbance of the butanol layer was measured at a wavelength of 532 nm. A calibration curve was constructed by running standards in triplicate.

**Peroxidase and eosinophil granule cytochemistry.** For peroxidase cytochemistry, ileal mucosa fixed in formalin was dehydrated with alcohol, cleared in xylene, and mounted in Permount. Tissue sections were incubated for 1 h at room temperature or overnight with 1:100 anti-IB4 rat IgG (ChromPure mouse immunoglobulin G [IgG], Jackson Immunoresearch Laboratories, West Grove, PA). IB4 antibodies abolished the ability of porcine neutrophils stimulated by treatment with the phorbol ester phorbol myristate acetate (data not shown). No adverse events were observed when uninfected piglets were treated identically with isotype control IgG or anti-CD18 antibody.

**In vivo inhibition of superoxide formation.** One-day-old littermate piglets were purified by litter weight and treated daily, beginning at the time of orogastric inoculation with 10^4 oocysts and continuing until 24 h prior to euthanasia on day 4 postinfection. Piglets were sedated by intramuscular injection of ketamine (10 mg/kg) and xylazine (1 mg/kg) prior to i.v. injection into a lateral ear vein. Superoxide formation was inhibited with treatment with superoxide dismutase-conjugated polyethylene glycol (PEG-SOD) (5,000 IU/pig i.v.; Sigma Chemical Co., St. Louis, MO) or an equivalent concentration of polyethylene glycol (PEG) alone (3.17 mg/pig i.v.). Drugs were reconstituted in phosphate-buffered saline (pH 7.4) and sterile filtered (0.22 μm) prior to injection.

**Immunohistochemistry for nitrotyrosine.** Immunohistochemistry for nitrotyrosine (NT) (1:200 polyclonal rabbit anti-NT; Cayman Chemical Co., Ann Arbor, MI) was performed using formalin-fixed, 5-μm sections of mucosal tissue. Tissues were treated with 3% H2O2 in methanol for 10 min at 4°C to quench endogenous peroxidase and blocked for 30 min at room temperature with non-immune goat serum. A commercial kit was used for blocking endogenous avidin and biotin activity (avidin/biotin blocking kit; Zymed Laboratories, Inc., San Francisco, CA). Tissue sections were incubated for 1 h at room temperature or overnight at 4°C. Sections were immunostained using a commercially available, broad-spectrum streptavidin-biotin-peroxidase system with DAB as the chromogen (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Sections were counterstained with methyl green. Negative-control sections were treated with isotype control primary antibody.

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had normal villus architecture and no evidence of epithelial infection. MDA, a by-product of lipid peroxidation, was significantly elevated in ileal mucosae from *C. parvum*-infected piglets (17.4 ± 1.8 nmol/mg protein for uninfected piglets \( n = 5 \); 36.3 ± 6.4 nmol/mg protein for infected piglets \( n = 6 \); \( P < 0.05 \)). Urinary excretion of \( F_2 \)-isoprostanes (free radical-catalyzed peroxidation products of polyunsaturated fatty acids [32]) was also increased in piglets infected with *C. parvum* (411 ± 121 pg of \( 8 \)-isoprostane/mg creatinine for uninfected piglets \( n = 6 \); 1,871 ± 773 pg of \( 8 \)-isoprostane/mg creatinine for infected piglets \( n = 5 \); \( P = 0.03 \)).

*C. parvum* infection results in neutrophilic inflammation. Human cryptosporidiosis is characterized by an influx of neutrophilic leukocytes into the intestinal mucosa (8, 9, 23). Mucosal infiltration of neutrophils has also been identified as characteristic of *C. parvum* infection in the present neonatal piglet model (3). To quantify neutrophilic infiltrates, ileal mucosae from uninfected control and infected piglets were assayed for activity of the neutrophil enzyme myeloperoxidase. Myeloperoxidase activity was significantly increased in ileal mucosae from *C. parvum*-infected piglets (activity per gram tissue was 7.6 ± 1.2 for uninfected piglets and 23.5 ± 3.0 for infected piglets \( n = 6 \) each; \( P = 0.002 \)). Peroxidase-positive cells and eosinophils were visualized in sections of ileal mucosa by means of histochemistry. In uninfected mucosa, peroxidase-positive cells were identified as eosinophils. In infected mucosa, peroxidase-positive cells were predominantly neutrophils admixed with fewer numbers of eosinophils (Fig. 1). As eosino-

![FIG. 1. Appearance of ileal mucosae from uninfected control and *C. parvum*-infected neonatal piglets examined by light microscopy after being stained for the presence of peroxidase-positive and eosinophil granule-containing cells. Control mucosae contained only peroxidase-positive eosinophils (arrows), while infected mucosae contained fewer numbers of eosinophils and numerous peroxidase-positive neutrophils. Peroxidase and eosinophil cytochemistry is shown for a single control and a single infected animal with a frozen and a paraffin-embedded sample, respectively.](image-url)
Antibody or PEG-SOD (significantly lowered by treatment of infected piglets with anti-CD18 vehicle, or (iv) PEG-SOD to scavenge superoxide. MPO activity was (ii) anti-CD18 antibody to inhibit neutrophil infiltration, (iii) PEG in infection. Piglets were treated daily with (i) isotype control antibody, (ii) anti-CD18 antibody to inhibit neutrophil infiltration, (iii) PEG oxidation activity, the numbers of eosinophils residing in control mucosa. When immunohistochemistry for localization of nitrotyrosine was performed on ileal mucosae from piglets treated with PEG-SOD (Fig. 2). Histochemical analysis of ileal mucosa from piglets treated with PEG-SOD revealed the presence of resident eosinophils only and not neutrophils. These results suggest that superoxide formation is a cause, rather than a consequence, of neutrophil influx in C. parvum-infected mucosa.

Peroxynitrite is generated independently of neutrophil influx in C. parvum infection. We have shown previously that the potent oxidant peroxynitrite is generated along the villus tips in C. parvum infection and in association with induction of inducible nitric oxide synthase (iNOS) expression (10). Peroxynitrite arises from the reaction of nitric oxide (NO) with superoxide radical. Neutrophils are the usual source of superoxide for peroxynitrite formation in vivo, and peroxynitrite formation is limited in a site-specific manner to areas of high superoxide generation (14). To determine whether neutrophils are the source of superoxide for peroxynitrite formation in C. parvum infection, we examined whether peroxidase-positive cells localize in proximity to the infected epithelium, where iNOS and protein tyrosine nitration (a footprint of peroxynitrite action) predominate. In mucosae from C. parvum-infected piglets, peroxidase-positive cells varied in location, from predominantly beneath the infected villus epithelium to predominantly within the lamina propria surrounding the crypts. When immunohistochemistry for localization of nitrotyrosine was performed on these same tissues, peroxynitrite formation remained greatest in proximity to the infected epithelial cells along the villus tips regardless of the variation in location of peroxidase-positive cells (Fig. 3). Further, inhibition of neutrophil influx did not attenuate NT formation by the intestinal mucosa of piglets infected with C. parvum.

Neutrophils do not contribute to control of epithelial infection or mucosal pathology in C. parvum infection. The impact of neutrophilic inflammation in limiting the extent of cryptosporidial infection is largely unknown. Therefore, it is of interest to determine whether inhibition of neutrophil influx alters the severity of epithelial infection or mucosal pathology. Neutrophil depletion of piglets infected with C. parvum had no significant effect on the severity of epithelial parasitism or villus atrophy (Table 1). There were also no differences in epithelial disruption between treatment groups (average percentage [±standard error] of villus denuded was 0 ± 0 for the uninfected control [n = 10], 6 ± 0.3 for the C. parvum plus isotype control group [n = 13], and 4 ± 0.1 for the C. parvum plus anti-CD18 group [n = 13]).
Neutrophil-derived mediators are not responsible for diarrhea in *C. parvum* infection. Neutrophils have been purported to be the source of a variety of mediators that may promote diarrhea in *C. parvum* infection. In infected piglets, neutrophil depletion had no effect on severity of diarrhea, maintenance of body weight (a parameter indicative of diarrheal severity in this model [11]) (Table 1), or intestinal epithelial short-circuit current (an indirect measure of Cl⁻ secretion in this tissue) (average μA cm⁻² [± standard error] was 40 ± 5.9 for the *C. parvum* plus isotype control group [n = 9] and 41 ± 4.6 for the *C. parvum* plus anti-CD18 group [n = 10]).

**Mucosal neutrophil influx promotes barrier function in *C. parvum* infection.** We have shown previously that *C. parvum* infection is associated with a decrease in paracellular barrier function (12). To determine whether neutrophils mediate physical or biochemical effects on barrier function in *C. parvum* infection, infected piglets were treated in vivo with isotype control or anti-CD18 antibody daily, beginning at the time of infection and continuing until peak infection severity (day 4), at which time mucosa from each piglet was mounted in Ussing chambers for measurement of TER and flux of ²²Na⁺ and [³H]mannitol. Inhibition of neutrophil influx decreased TER significantly, indicating a decrease in barrier function of the infected mucosa (Fig. 4). Transepithelial flux of ²²Na⁺ and [³H]mannitol was also increased in mucosae from piglets treated with anti-CD18; however, sample sizes did not allow

![Image](https://iai.asm.org/5501/figure3.jpg)

**FIG. 3.** Immunohistochemistry for tyrosine-nitrosated proteins (nitrotyrosine, a stable “footprint” of peroxynitrite action) was performed using ileal mucosae obtained from *C. parvum*-infected piglets having a mild pericryptal peroxidase-positive cellular infiltrate (A) or a predominantly subepithelial peroxidase-positive cellular infiltrate (B). Peroxynitrite formation was greatest in proximity to infected enterocytes along the apical villus regardless of the variation in location of peroxidase-positive cells or apparent degree of epithelial infection. The counterstain used was methyl green. H & E, hematoxylin and eosin.
TABLE 1. Indices of severity of C. parvum infection in the ilea of neonatal piglets treated with anti-CD18 antibody or isotype control antibody (IgG)*

<table>
<thead>
<tr>
<th>Treatment group (n)</th>
<th>Villus ht (μm)</th>
<th>Crypt depth (μm)</th>
<th>No. of epithelial cells per villus</th>
<th>No. of parasites per villus</th>
<th>No. of parasites per epithelial cell</th>
<th>Body wt (kg) at peak infection*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected control (10)</td>
<td>638 ± 39</td>
<td>133 ± 8</td>
<td>208 ± 14</td>
<td>0</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td>C. parvum plus IgG (13)</td>
<td>204 ± 33</td>
<td>142 ± 6</td>
<td>106 ± 17</td>
<td>49 ± 9</td>
<td>0.7 ± 0.2</td>
<td>3.05 ± 0.26*</td>
</tr>
<tr>
<td>C. parvum plus anti-CD18 (13)</td>
<td>241 ± 44</td>
<td>130 ± 8</td>
<td>118 ± 19</td>
<td>53 ± 13</td>
<td>0.7 ± 0.2</td>
<td>3.08 ± 0.30*</td>
</tr>
</tbody>
</table>

* Daily treatments began at time of infection and continued until euthanasia at peak infection (day 4). Values are means ± standard errors. P values are as follows: for villus height, 0.16; for crypt depth, 0.44; for number of epithelial cells per villus, 0.30; for number of parasites per villus, 0.37; for number of parasites per epithelial cell, 0.48; and for body weight at peak infection, 0.37.

For assay of mucosal PGE2 synthesis, sheets of ileal mucosa from anti-CD18 antibody- or isotype control antibody-treated piglets were mounted in Ussing chambers. No significant difference in PGE2 synthesis between anti-CD18 and isotype control groups was observed with ileal mucosae obtained from uninfected control piglets and those infected with C. parvum plus anti-CD18 antibody (Fig. 4, inset). Antisera against C. parvum 18S rRNA or C. parvum 28S rRNA were used for RNA analysis.

Infection of neonatal piglets with C. parvum resulted in significant recruitment of neutrophils to ileal mucosa as determined by cytochemistry and increases in the activity of the neutrophil enzyme myeloperoxidase. Myeloperoxidase activity has been used extensively as a means to quantify neutrophil infiltration in intestinal mucosa. Eosinophils may also contribute to measures of MPO activity; however, the number of eosinophils present in ileal mucosa was significantly less in infected than in control mucosa. Neutrophil infiltration into C. parvum-infected mucosa was entirely inhibited by treating piglets with anti-CD18 antibody insofar as MPO activity was reduced to levels observed for uninfected control piglets and neutrophils were not observed in histologic sections. Residual MPO activity measured in mucosa from uninfected piglets and those treated with anti-CD18 was attributed to the presence of resident, peroxidase-positive eosinophils, whose identity was established by cytochemistry.

The mechanism by which a noninvasive epithelial pathogen such as C. parvum elicits subepithelial inflammation is not well understood. In xenografts of human intestine and model epithelium, C. parvum infection results in expression of the chemokines interleukin-8, tumor necrosis factor alpha (TNF-α), and GROα (7, 16, 21, 22, 25, 38). These chemokines are hypothesized to signal neutrophil influx into intestinal mucosa in C. parvum infection. Superoxide has been identified as a key mediator of neutrophil recruitment in intestinal injury induced by reperfusion, irradiation, dextran sodium sulfate, acetic acid, and trinitrobenzene sulfonic acid (13, 19, 27, 37, 40, 42). Because neutrophil depletion did not attenuate lipid peroxidation of mucosa from C. parvum-infected piglets, we considered the possibility that neutrophil recruitment was mediated by superoxide. Accordingly, treatment of C. parvum-infected piglets with superoxide dismutase inhibited the rise in mucosal MPO activity resulting from C. parvum infection, demonstrating that neutrophil recruitment was mediated by superoxide-dependent mechanisms. Thus, formation of superoxide is identified here as a cause, rather than a sole consequence, of neutrophilic inflammation in C. parvum-infected intestine. Probable cellular sources of superoxide for neutrophil recruitment in C. parvum infection include activated macrophages (28), intestinal epithelium, or vascular endothelium. A significant influx of macrophages has been demonstrated in the present model (17), and peroxynitrite, a reaction product of nitric oxide and superoxide, is generated at epithelial sites in C. parvum-infected mucosa (10). Inhibition of peroxynitrite formation is unlikely to be the means by which SOD inhibited neutrophil influx into C. parvum-infected mucosa, however, as superoxide reacts with NO at rates that exceed dismutation by SOD (31). It has been shown with patients with ulcerative colitis that increased superoxide production can be attributed to both invading monocytes and the vascular endothelium (30). In vascular endothelium, cytokines induce superoxide-dependent expression of adhesion molecules, such as ICAM-1, which increase the transmigration of neutrophils into inflamed intestine (5, 27, 31, 34). Importantly, our finding that superoxide formation is required for neutrophil recruitment in C. parvum infection does not negate the importance of epithelial-derived cytokines and chemokines in initiating activation of those lamina propria cells responsible for superoxide formation. Such a role has already been demonstrated for TNF-α (5, 34).
relatively modest neutrophilic inflammation observed with *C. parvum* compared to enteroinvasive infection may be attributed to the enhanced ability of the intestine to dismutate superoxide and the absence of xanthine oxidase activity in young piglets (6).

We have shown previously that peroxynitrite is generated along the villus tips in *C. parvum* infection and in association with induction of epithelial iNOS expression (10). Peroxynitrite arises from the diffusion-limited reaction of superoxide radical with NO. Because the cellular concentration of superoxide is 1,000 times less than that of NO, peroxynitrite formation is limited in a site-specific manner to areas of high superoxide generation (14). Therefore, we examined whether peroxidase-positive cells, as putative sources of superoxide, localize in proximity to the infected epithelium, where iNOS and protein tyrosine nitration (a footprint of peroxynitrite ac-

![Graph showing TER and permeability of C. parvum-infected ileal mucosae](image)

**FIG. 4.** TER and permeability of *C. parvum*-infected ileal mucosae from neonatal piglets treated with anti-CD18 antibody to inhibit neutrophil influx and activation or isotype control antibody. Mucosae were mounted in Ussing chambers and TER recorded over a 120-min period. Passive flux of $^{22}$Na$^+$ from serosa to mucosa ($J_{sm}$) and passive flux of [H]mannitol from mucosa to serosa ($J_{ms}$) were measured between the time points of 60 and 120 min. TER of mucosae from neutrophil-depleted piglets was significantly lower than that for piglets treated with isotype control antibody (*, $P < 0.05$ by Friedman repeated-measures analysis of variance on ranks), and this finding was supported by an increase in flux of $^{22}$Na$^+$ and [H]mannitol. A significant decrease in TER was not observed in ileal mucosae obtained from uninfected control piglets treated identically with isotype control or anti-CD18 antibody (inset).
tion) predominate. Our results demonstrate that neutrophils are not consistently found in proximity to the infected epithelium, nor are they required for peroxynitrite formation. These observations suggest that superoxide arises from another cell type residing near the villus tips, such as macrophages or intestinal epithelium. Efforts were made, using five different antibodies, to determine the location of macrophages along the villus-crypt axis of control and infected mucosas; however, unambiguous staining of cells could not be obtained. As a consequence, any effect of anti-CD18 antibody treatment on macrophage infiltration cannot be determined by the present study.

It is of interest to determine whether inhibition of neutrophil influx alters the severity of epithelial infection or mucosal pathology. In AIDS patients with cryptosporidiosis, no correlation between neutrophilic inflammation and intensity of infection was found (23). On the other hand, antioxidants have been shown in several studies to exacerbate C. parvum infection (10, 15, 44). Whether neutrophils are the source of these antiparasitic oxidants has not been determined. In the present study, neutrophil depletion had no effect on severity of C. parvum infection. In addition to the lack of effect of neutrophil depletion on measures of lipid peroxidation and peroxynitrite formation, these findings suggest that neutrophils are not a significant source of free radicals in C. parvum-infected mucosa. This may be attributed to a paucity of neutrophil activation factors released as a consequence of the minimally invasive infection. For example, in studies of transgenic mice, the induced expression of interleukin-8 by intestinal epithelium is alone sufficient to recruit neutrophils into intestinal mucosa; however, they do not become activated or result in intestinal injury (20). Results of the present study do not identify the cellular source of peroxidative injury in C. parvum infection, although activated macrophages are a likely possibility.

The mechanism(s) by which C. parvum infection results in diarrhea remains speculative. Diarrhea has been attributed to villus atrophy-associated malabsorption and to mucosal inflammation-induced alteration in intestinal water and electrolyte transport. Neutrophils are a source of mediators, including reactive oxygen metabolites (4), TNF-α (17), 5'-AMP (24), and prostaglandins (1, 2, 4, 12), that may directly or indirectly promote epithelial secretion and diarrhea in C. parvum infection. Neutrophil depletion of C. parvum-infected piglets had no effect on clinical severity of diarrhea or epithelial Cl− secretion, suggesting that mediators derived from or stimulated by neutrophils do not play a significant role in the genesis of diarrhea in the infection.

Although neutrophilic inflammation was not responsible for severity of villus atrophy or loss of epithelial continuity in C. parvum-infected mucosa, neutrophils may biochemically and physically compromise intestinal barrier function by disrupting epithelial tight junctions (29). We have shown previously that C. parvum infection results in a loss of barrier function that is compensated for by an increase in mucosal synthesis of prostaglandins (12). To determine if neutrophils are the cause of this defect in barrier function, transepithelial electrical resistance and flux of 22Na and [3H]mannitol were measured using ileal mucosae from neutrophil-depleted and isotype control-treated piglets infected with C. parvum. Barrier function was significantly worsened, rather than improved, by the absence of neutrophils. This effect could not be attributed to a deficiency in mucosal prostaglandin synthesis. Perhaps more interesting than the barrier-sparing effect of neutrophils is the fact that neutrophil depletion had no effect on mortality, mucosal injury, or clinical severity of disease. These observations suggest that barrier function is well-maintained in C. parvum infection and that the barrier-sparing effects of neutrophils may not be clinically significant. The latter is suggested by the sustained ability of the mucosal epithelium from anti-CD18 antibody-treated piglets to restrict permeation by [3H]mannitol. Although the cause of the barrier-sparing effect of neutrophils is not disclosed by the present study, neutrophils may mediate this effect by stimulating the release of cytokines that modulate tight junction function or by removing factors that contribute to loss of barrier integrity.

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