Cryptosporidiosis-Induced Impairment of Ion Transport and Na\(^+\)-Glucose Absorption in Adult Immunocompromised Mice

Nathalie Kapel, Jean Francois Huneau, Denis Magne, Daniel Tomé, and Jean-Gérard Gobert

Electrolyte transport was investigated during chronic cryptosporidiosis in adult anti–interferon-γ–treated SCID mice by means of Ussing chamber techniques. In basal conditions, infection of immunocompromised mice with *Cryptosporidium parvum* resulted in a 30% reduction (P < .05) in the ileal short-circuit (Isc) current related to a 28% reduction (P < .05) in tissue conductance compared with controls. The rises in Isc and transepithelial potential difference induced by glucose (10 mM) were significantly reduced by *Cryptosporidium* infection (P < .01) compared with controls. In contrast, responses to mucosal glutamine were marginally affected. Electrical parameters of the ileum were not affected by the addition of indomethacin or furosemide, in either control or *Cryptosporidium*-infected mice. Thus, long-term cryptosporidiosis in immunocompromised animals leads to a reduction in net ion exchanges, decreased paracellular shunting, and impaired Na\(^+\)-glucose cotransport in the ileum, without prostanoid- or enterotoxin-mediated electrogenic Cl\(^-\) secretion.

*Cryptosporidium parvum* is a coccidial protozoan that infects the gastrointestinal epithelial cells of humans and other mammals. The infection can involve all of the gastrointestinal segments but is mainly located in the ileum [1]. In immunocompetent persons, *C. parvum* infection is uncommon and causes self-limited diarrhea. In contrast, in patients with AIDS, cryptosporidiosis is a frequent opportunistic infection causing unremitting and frequently life-threatening diarrhea [1]. The complex pathophysiology of cryptosporidiosis has been studied mainly in immunocompetent models. Human and animal data suggest that *C. parvum* infection induces a malabsorptive diarrheal syndrome caused through damage to cells of the villus tip associated with morphologic alterations of the intestinal epithelium and impaired glucose-stimulated Na\(^+\) absorption [2, 3]. Further studies indicated that the cryptosporidiosis diarrhea is in part of secretory origin. Using a colostrum-deprived neonatal piglet model of cryptosporidiosis, Argenzio et al. [4] detected prostaglandin-mediated secretion of Cl\(^-\). They have also shown that glutamine enhances electrogenic as well as neutral Na\(^+\) absorption by a mechanism that involves prostaglandin-sensitive, Cl\(^-\)-dependent, apical Na\(^+\)/H\(^+\) exchanges [5]. An enterotoxic effect of *Cryptosporidium* mediated by a heat-labile, calcium-dependent, reversible active factor leading to chloride secretion has also been shown [6].

Many of these data have been obtained during self-limited cryptosporidiosis in neonatal models. The purpose of this study was to investigate electrolyte transport in basal conditions, glucose- and glutamine-dependent NaCl transport during experimental chronic cryptosporidiosis. An adult anti–interferon (IFN)-γ–treated SCID mouse model of cryptosporidiosis was chosen because these animals develop chronic cryptosporidiosis with abundant oocyst shedding [7].

**Materials and Methods**

*Animals and housing.* SCID mice (CB-17 scid/scid) were obtained from IFFA CREDO (l’Arbresle, France) and housed in filter-topped microisolator cages in an air-filtered cupboard. They were given sterile food and water, and their cages and bedding were exchanged every week for sterilized ones. They were 6 weeks old at the start of the experiment. Their phenotypic purity was confirmed by the absence of serum IgM.

*C. parvum* was produced in a high-yield outbred suckling mouse model [8]. Fifteen SCID mice were challenged by gastric intubation with 400 μL of mouse colonic perfusate containing 10⁷*Cryptosporidium* in 0.025 M PBS, pH 7.2. Ten SCID mice inoculated by gastric intubation with 400 μL of colonic perfusate containing no *cryptosporidia* were used as controls. One day before inoculation, all mice received an intraperitoneal injection of 125 μg of hamster monoclonal anti-murine IFN-γ. Because this antibody has a half-life of ~1 week, the mice received an additional 62.5 μg of antimurine IFN-γ 1, 2, and 3 weeks after the initial treatment [7]. Parasite load was checked each week until the day before Ussing chamber studies by counting cryptosporidia in 30 high-power microscopic fields (×200) of modified acid fast–stained fecal smear.
Ussing chamber studies. At week 4 after inoculation, infected and control mice were killed by cervical dislocation and the ileum was removed. Two adjacent segments were rinsed free of intestinal contents, opened along the mesenteric border, and mounted in an Ussing chamber (Marty Technologie, Marcilly sur Eure, France). The mucosal and serosal compartments of the chambers were filled with 4 mL of Ringer’s solution (pH 7.4) containing 140 mM Na⁺, 5.2 mM K⁺, 1.2 mM Ca²⁺, 1.2 mM Mg²⁺, 120 mM Cl⁻, 2.4 mM HPO₄²⁻, and 0.4 mM H₂PO₄⁻ and supplemented with 5 mM sodium pyruvate. Oxygenation of the tissue was ensured by a gas lift of O₂/CO₂ (95:5), and the temperature was maintained at 37°C throughout the experiment. The tissue was short-circuited by an automatic voltage clamp (World Precision Instruments, New Haven, CT). The transepithelial potential difference (PD), measured by using calomel electrodes, and the short-circuit current (Isc) were continuously monitored. Tissue conductance (G) was calculated according to Ohm’s law.

After electrical parameters were stabilized for at least 15 min, Isc, PD, and G were recorded twice at a 5-min interval, and the mean was used as the basal value. Thereafter, for each sample, glucose (final concentration: 10 mM) was added to the mucosal compartment of the first chamber and glutamine (final concentration: 10 mM) was added to the mucosal compartment of the second chamber. The maximal deviation of Isc, PD, and G resulting from glucose and glutamine addition was measured to determine the absorption rate of the nutrients [9]. After 15 min, 10 μM indomethacin or 10 μM furosemide (Sigma, St. Louis) was added to the two compartments to check for possible involvement of prostaglandins and the Na⁺/K⁺/2 Cl⁻ cotransport system in the changes in electrophysiologic parameters during cryptosporidiosis.

Statistical analysis. For each animal, Isc, PD, and G values are the means of two determinations recorded in the two adjacent segments. Results are reported as means ± SEs of three and four experiments performed with controls and infected animals, respectively. Statistical analysis was done with Student’s t test for unpaired data.

Results

Animal model. Infection in immunocompromised mice became patent during week 2 (mean number of oocysts per field, ≤1), and there was a rapid increase in oocyst shedding during the next two weeks (parasite score from 1+ [1 oocyst per field, week 3] to 3+ [≥20 oocysts per field, week 4]). None of the anti-IFN-γ–treated mice exhibited clinical manifestations of cryptosporidiosis—diarrhea, wasting, or weight loss—during the study period. No oocysts were detected in control mice.

In vitro electrical studies. In basal conditions, infection of immunocompromised mice with Cryptosporidium organisms resulted in a significant reduction in the ileal Isc (36.6 ± 3.6 μA/cm²) compared with controls (52.3 ± 2.5 μA/cm²; P < .01) and a significant reduction in G (36.7 ± 4.8 mS/cm² vs. 26.4 ± 1.5 mS/cm² for control and infected animals, respectively; P < .05), whereas the transepithelial PD was only marginally affected (−1.88 ± 0.13 mV vs. −1.57 ± 0.19 mV for control and infected animals, respectively).

Following addition of 10 mM glucose or 10 mM glutamine to the mucosal side of the ileum, Isc and PD rose in both Cryptosporidium–infected and control mice, but neither glucose nor glutamine affected G (figure 1). The rise in Isc induced by glucose was significantly reduced by Cryptosporidium infection (ΔIsc = 20.0 ± 2.3 μA/cm² vs. ΔIsc = 11.6 ± 1.3 μA/cm² for control and infected animals, respectively; P < .01). After glucose addition, the PD deviation was less in Cryptosporidium–infected mice compared with controls, and both glucose and glutamine caused a significant drop in PD (figure 1).

Figure 1. Maximal changes in ileal short-circuit current (Isc), transepithelial potential difference (PD), and tissue conductance (G) induced by 10 mM glucose or 10 mM glutamine in ileal mucosa. Results are expressed as means ± SE. * P < .05; ** P < .01.
Discussion

Although *C. parvum* is an important cause of diarrhea in immunocompetent and immunocompromised hosts, the pathophysiology of the infection is poorly understood. Here we report that anti–IFN-γ–treated SCID mice develop mild chronic *C. parvum* infection. In the ileum, cryptosporidiosis was associated with a reduced short-circuit current and tissue conductance, and impaired Na⁺-glucose cotransport, without prostanooid-mediated or enterotoxic electrogenic Cl⁻ secretion.

In contrast with immunocompetent mice, adult anti–IFN-γ–treated SCID mice develop chronic infection with patent histopathologic abnormalities 4 weeks after inoculation, even though there are no clinical manifestations [7]. We thus measured epithelial electrophysiologic parameters and nutrient transport in ileal mucosa from control and *C. parvum*–infected SCID mice, by use of Ussing chambers. Our results indicate that in basal conditions, cryptosporidiosis leads to a significant reduction in ileal short-circuit current and conductance, suggesting that both net ion exchanges across the epithelium and the paracellular shunt are reduced. This last observation contrasts with results from in vitro studies showing that the electrical resistance of epithelial cell monolayers decreases as a consequence of *C. parvum* infection [10]; however, it concords with results of studies in neonatal piglets indicating that a reduction in ileal conductance, that is, an increase in ileal resistance, occurs during acute cryptosporidiosis [3, 4]. Tissue conductance is assumed to reflect solute permeation across tight junctions and has been shown to be regulated by the cytoskeleton [11]. In enterocytes, *Cryptosporidium* induces a rearrangement of the cell cytoskeleton, resulting in the formation of a dense band of modified host cytoskeleton proteins underlining the trophozoite-containing vacuoles, which may serve to anchor the parasite to the host cell or, alternatively, resist further invasion into the absorptive cell cytoplasm [12]. This alteration of the host cell cytoskeleton could affect the permeability of the junctional complex and therefore account for the decreased paracellular shunting observed during cryptosporidiosis.

Another important feature of our results is the reduction in the glucose-induced increase in Isc in *C. parvum*–infected mice relative to uninfected animals, suggesting that chronic cryptosporidiosis impairs Na⁺-glucose absorption in immunocompromised mice. In contrast, Na⁺-glutamine cotransport was only marginally affected. A reduction in ileal Na⁺-glucose cotransport has been reported shortly after *C. parvum* challenge in unweaned piglets [2–4]. As the Na⁺-glucose cotransport is mainly expressed in the quiescent enterocytes of the upper half of the intestinal villi [13], reduced villus height is likely to result in smaller number of epithelial transporters and, therefore, in impaired Na⁺-glucose absorption. In contrast, Na⁺-glutamine cotransport appears to occur in differentiated enterocytes as well as in proliferating undifferentiated epithelial cells [14]. This difference could explain why the epithelial response to mucosal addition of glutamine was preserved in our study while glucose absorption was impaired. Such a result has also been reported by Argenzio et al. [5] during acute cryptosporidiosis. Therefore, glutamine could be more effective than glucose in promoting oral rehydration during cryptosporidial diarrhea.

Contrasting with results obtained in neonatal piglets, chronic cryptosporidiosis in immunocompromised mice was not associated with diarrhea. In newborn piglets, Argenzio et al. [4] have shown that an inhibition of ileal NaCl absorption, arising from stimulation of prostanoid synthesis, occurs during acute cryptosporidiosis. This reduction in NaCl absorption probably plays a major role in *C. parvum*–induced diarrhea. With our model, we failed to observe any involvement of prostaglandins in the alteration of the short-circuit current in *C. parvum*–infected IFN-γ–depleted mice. Marked interspecies variability in the production and involvement of eicosanoids in physiologic and pharmacologic control of intestinal ion transport are known to exist [15] and may account for the discrepancies between our results and those from Argenzio et al. [4]. Moreover, the use of an immunocompromised animal model may also contribute to these differences, as immune cells in the lamina propria are

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<th>Mouse group</th>
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<td></td>
<td>ΔIsc (µA/cm²)</td>
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<td>Control (n = 6)</td>
<td>−4.7 ± 2.3</td>
<td>0.17 ± 0.6</td>
<td>0.47 ± 0.61</td>
<td>2.7 ± 1.3</td>
<td>0.07 ± 0.03</td>
<td>−2.37 ± 1.12</td>
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<td><em>C. parvum</em>–infected (n = 10)</td>
<td>−1.3 ± 0.7</td>
<td>0.08 ± 0.02</td>
<td>0.50 ± 0.23</td>
<td>−0.2 ± 1.6</td>
<td>0.05 ± 0.02</td>
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NOTE. Data are means ± SEs. Isc, ileal short-circuit current; PD, transepithelial potential difference; G, tissue conductance.
usually considered to be the main source of eicosanoids in the intestinal mucosa [15].

At present, most experiments on the pathophysiology of C. parvum—induced diarrhea have been done in piglets or calves, as the different attempts to create a rodent model have failed. Our results indicate that although cryptosporidiosis leads to an impairment of ion transport and Na⁺-glucose absorption, it does not trigger diarrhea in IFN-γ depleted mice. This failure is unlikely to arise from reduced intestinal manifestation in mice compared to piglets or calves. Indeed, a large number of oocysts was observed in fecal smears of anti-IFN-γ–treated SCID mice at week 4 after infection. Rather, it seems that a difference in the host response to a similar parasite load is responsible for the differences in the pathophysiology of cryptosporidiosis between mice and piglets. As suggested above, part of this difference may lie in the way epithelial and subepithelial cells produce prostaglandins in response to C. parvum infection.

In conclusion, we provide evidence that chronic cryptosporidiosis in immunocompromised adult mice impairs Na⁺-glucose cotransport and alters net electronegic ion transport across the intestinal epithelium. Although not reproducing all the symptoms occurring during chronic cryptosporidiosis in immunocompromised patients, this model represents a useful tool to investigate the pathophysiology of C. parvum infection.

References