**NOTE** Parasitology

**Murine Goblet Cell Hypoplasia during *Eimeria pragensis* Infection is Ameliorated by Clindamycin Treatment**

Muchammad YUNUS\(^1\), Yoichiro HORII\(^1\), Susumu MAKIMURA\(^1\)* and Adrian L. SMITH\(^2\)

\(^1\)Laboratory of Veterinary Internal Medicine, Department of Veterinary Science, Faculty of Agriculture, University of Miyazaki, Gakuen-Kibanadai Nishi 1–1 Miyazaki 889–2192, Japan and \(^2\)Enteric Immunology Group, Division of Immunology and Pathology, Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berkshire RG20 7NN, United Kingdom

(Received 27 July 2004/Accepted 5 November 2004)

**ABSTRACT.** The goblet cell (GC) and the intestinal mucus are important in preventing invasion of the mucosa by luminal microorganisms. GC responses in the jejunum, cecum and colon of C57BL/6 mice during the course of infection with the large intestinal-tropic coccidian, *Eimeria pragensis* (*E. pragensis*), were investigated histologically. The numbers of large intestinal GCs (cecum and colon) gradually decreased (hypoplasia) in association with development of endogenous stages of parasite life cycle. The effect was transient and recovery of GC numbers was associated with resolution of coccidial infection. The jejunal GC numbers were not affected by *E. pragensis* infection. Clindamycin treatment in the infected mice reduced numbers of intracellular parasites and significantly increased the numbers of large intestinal GCs compared with untreated, infected mice.

**KEY WORDS:** clindamycin, *E. pragensis*, hypoplasia of goblet cell.

The goblet cells (GCs) are specialized epithelial cells that constitutively produce a protective mucus layer to protect the epithelium and also respond to pathologic challenge by increasing the rate of mucus production and by changing the constituents of the mucus [12, 18]. The eimerian parasites represent the principal pathogens of the intestinal tract in poultry and many other domestic animals, invading and destroying epithelial cells leading to the clinical disease, intestinal coccidiosis. Although each *Eimeria* spp. is highly host specific almost all vertebrate species (with the exception of humans and primates) can be infected with eimerian parasites and these interactions may be significant in terms of the evolution of the vertebrate enteric defense system. Clinical coccidiosis involves dramatic remodeling of the mucosa with extensive damage that leads to reduced absorption, hemorrhage and fluid leakage from damaged mucosa that is also associated with a dynamic immune and inflammatory response [2]. Sub-clinical infection will result in localized damage to the epithelia offering the potential for invasion by gut dwelling microorganisms such as the opportunistic pathogen *Clostridium perfringens*. Indeed, infection with eimerian parasites has been associated with increase in the incidence of clostridial necrotic enteritis in poultry [21]. Since these interactions have significant impact on the livestock industry, we characterized the GC response in mice inoculated with *E. pragensis*, that infects enterocytes in the cecum and colon and shares many biological characteristics with the important pathogen of chickens, *E. tenella* [15]. On the other hand, clindamycin is active against apicomplexan parasites such as *Plasmodium*, *Toxoplasma*, *Babesia*, and the fungal pathogen *Pneumocystis* [9]. Prophylactic application of anticoccidial drugs represents the major mechanism of coccidiosis control. Clindamycin treatment has been successfully used to reduce the load of *E. pragensis* [19]. The study was designed to assess the response of GC at the site of parasite development (cecum and colon), proximal to the site of *E. pragensis* development (jejunum) in infected mice, and by clindamycin treatment.

**Animals and parasites:** Female C57BL/6 mice, 8–9 weeks old with around 25 g body weight (BW) were purchased from Clea & Shizuoka Experimental Animal Supplier Co., Japan, housed in clean metal cages and fed with a standard diet and tap water *ad libitum* in an air-conditioned room (23 ± 1°C), under conventional conditions with a 12:12 hr, light: dark cycle. They were kept as outlined in the “Guide for the Care and Use of Laboratory Animals” by The Faculty of Agriculture, University of Miyazaki. The pathogenic agent used in this study, *E. pragensis* [3] originated from Dr Dawn Owen (MRC) and has been maintained in the Compton laboratory for over 20 years by oral passage in C57BL/6 mice [11].

**Drug:** The drug, clindamycin phosphate "Dalacin S" (Pharmacia Co., Tokyo) administered at a dose of 800 mg/kg/day in distilled water, by subcutaneous injection with a treatment schedule of daily injections from day 4 to day 8 post infection (pi). This treatment schedule reduces output of oocysts from *E. pragensis* infected mice by sixteen folds [19].

**Histological examination:** Samples of jejunum, cecum and colon were taken from infected and uninfected mice, fixed in 10% neutral buffered formalin overnight followed by the stratified dehydration in alcohol and embedding in the paraffin wax. Serial 4 μm thick sections were cut, deparaffinized, rehydrated and stained with hematoxylin and eosin for routine histology, or with Alcian blue and periodic
produced with the C57BL/6 mice used in this study was approximately 7 to 8 days pi. The total numbers of oocysts in the cecal and colonic epithelia were in largest numbers at rapid rates and the last oocysts were detected on 11–12 days pi. reached peak on the 8–9 days pi before numbers declined pragensis those previously reported [10, 14] with this isolate of presented as mean ± standard deviation (SD).

Experimental procedures: Experiment 1: This experiment was performed to observe response of intestinal GCs to E. pragensis infection in mice. Forty mice were infected orally through a gastric tube with 1 × 10^6 E. pragensis sporulated oocysts in 0.1 ml distilled water [19], and the day of infection was defined as day 0 (uninfected control). Five mice were killed every 2 days from day 0 to day 14 pi using GC examination. The last 5 mice were used for GCs and the daily oocyst output examination. The daily oocyst output was counted by McMaster chamber method from day 7 to day 12 pi. Experiment 2: This experiment was carried out to evaluate the effect of chemotherapy with clindamycin on the response of intestinal GCs to E. pragensis infection in mice. Twenty mice were divided into 4 groups. 1) Uninfected mice injected distilled water, 2) uninfected mice injected clindamycin, 3) infected mice injected distilled water and 4) infected mice injected clindamycin. Mice were infected orally through a gastric tube with the same doses of E. pragensis (experiment 1). Clindamycin was injected subcutaneously into the back part of body at 24 hr intervals from day 4 to day 8 pi, a schedule that inhibits the development of E. pragensis [19]. Intestinal GCs were counted on the 9th day pi for each group, which E. pragensis infection at peak level of the daily oocyst output. Effect of drug was expressed by the mean of the number of GCs. Results were statistically analyzed using student’s t-test and a p value below 0.05 was considered significant. All results are presented as mean ± standard deviation (SD).

The temporal pattern of oocyst output per day confirms those previously reported [10, 14] with this isolate of E. pragensis. Oocysts first appeared on the 6–7 days pi, then reached peak on the 8–9 days pi before numbers declined rapidly and the last oocysts were detected on 11–12 days pi. The numbers of intracellular parasitic stages present within the cecal and colonic epithelia were in largest numbers at approximately 7 to 8 days pi. The total numbers of oocysts produced with the C57BL/6 mice used in this study was [19.9 ± 5.2] × 10^6/mouse and the period of patency was [12.4 ± 0.7] days (Fig. 1).

Infection was associated with various histopathological changes in the cecum and colon but not in the jejunum of infected mice. The histopathology included substantial hyperplasia of the crypt enteroocytes, damage to the surface mucosa and extensive infiltration in the lamina propria, most evident in the colon of infected mice. Numerous intracellular parasites were associated with these histopathological lesions (Fig. 2). There were dramatic changes in the numbers of GC present in the cecum or colon of infected mice but no changes in the number of jejunal GC (Figs. 1 and 2). The numbers of cecal and colonic GC were decreased from 2 days pi and continued to decrease until 8 days pi (Fig. 1). At 8 days pi the numbers of large intestinal GC were approximately five folds less numerous than with uninfected (day 0) mice. Rapid recovery of GC numbers in the large intestine occurred after 10 days pi and by 12–14 days pi the numbers had returned to normal (colon) or near normal (cecum) levels (Fig. 1). The numbers of GC in the jejunum were unaffected by infection of mice with E. pragensis (Figs. 1 and 2).

To determine whether anti-coccidial therapy affected the GC hypoplasia associated with infection mice were treated with therapeutic doses of clindamycin [19] and GC numbers were assessed by histological examination of gut sections at 9 days pi. The clindamycin treatment schedule reduced oocyst output from [22.6 ± 5.1] × 10^6/mouse to [1.4 ± 0.6] × 10^6/mouse. These reductions in parasite numbers also lessened the intensity of histopathological lesions in treated mice [19]. The numbers of jejunal GCs per villus crypt unit were unaffected by either infection or by treatment with clindamycin (Fig. 3). Consistent with the data presented in Figs. 1 and 2, infection of mice with E. pragensis reduced the number of GC in both cecum and colon by a substantial amount (approximately eleven folds; Fig. 3). Treatment of uninfected mice with clindamycin for 5 days had no effect on the GC numbers in jejunum, cecum or colon. However, treatment of infected mice with clindamycin diminished the effect of infection on GC numbers in both cecal and colonic locations of the intestine (Fig. 3). The numbers of cecal GCs per 10 crypt units (GC/10CU) of infected, untreated
mice (22.0 ± 4.5 GC/10CU/mouse) and infected, treated mice (69.6 ± 5.0 GC/10CU/mouse) were significantly lower than seen with untreated or treated, uninfected animals (117.8 ± 10.3 GC/10CU/mouse and 110.6 ± 9.9 GC/10CU/mouse respectively; p<0.001). More importantly, in mice infected with *E. pragensis*, the numbers of cecal and colonic GCs were significantly higher in the clindamycin treated group than with the untreated group (Fig. 3b and 3c; p<0.001). In the cecal tissue of infected mice, the number of GC increased from 22.0 ± 4.5 GC/10CU/mouse to 69.6 ± 5.0 GC/10CU/mouse after clindamycin treatment (p<0.05). Similarly, in the colonic tissue of clindamycin treated infected mice, the number of GC increased from an untreated level of 10.2 ± 1.9 GC/10CU/mouse to 97.2 ± 7.2 GC/10CU/mouse (p<0.001). The number of colonic GCs in uninfected, untreated and uninfected, treated groups were 180.4 ± 13.4 GC/10CU/mouse and 168.8 ± 10.2 GC/10CU/mouse, respectively, both being significantly different to the infected groups (p<0.001) but not significantly different from each other (p>0.05).

In the present experiments, even low dose of *E. pragensis*-oocysts (1 × 10²) induced significantly clinical eimeriosis, but no mortality [19], and the parasites predominantly invaded the crypt region of the cecal and colonic epithelia, although mature gametocytes/zygotes were present in surface epithelial cells (Fig. 2). This type of pathology is typical of those coccidial parasites that invade the large intestinal epithelia [6] and the host inflammatory response appears to be responsible for at least some of these changes [16]. Indeed, with *E. nieschulzi* (a small intestinal tropic parasite), the damage to the epithelial surface was greatly reduced in T cell-deficient nude rats despite a dramatic increase in the numbers of parasites developing in the gut [13]. A range of changes in the immune cell populations associated with coccidial lesion has been reported [6, 16], including intraepithelial lymphocyte populations [20], mast cells [7, 13] and polymorphonuclear cells [20].

The dramatic change in GC numbers was limited to the site of infection and not evident in the jejunum, which lies proximal to the developmental site of *E. pragensis*. Since the *E. pragensis* parasites develop in the crypt region that contains the multipotential stem cells [5], the reduction in
GC may reflect damage to the stem cell population. Indeed, GCs arise by mitosis from multipotential stem cells at the base of the crypt [5] and the depletion of GC was most evident in infected crypts and much less in neighboring uninfected areas of the intestine. Changes in GC numbers may affect the susceptibility of the parasite-infected host to limit the capacity of opportunistic pathogens from interacting or penetrating the local epithelium. Indeed, subclinical coccidiosis is a predisposing factor in the development of necrotic enteritis under experimental or field conditions [1, 21].

The use of anticoccidial drugs is the main method for the control of coccidiosis in the field and we used clindamycin treatment to reduce parasite load [19] and monitored the effects on GC numbers. Treatment was associated with significant recovery of GC count of the large intestinal mucosa of infected mice when compared with unmedicated infected mice. Jejunal GC numbers were not influenced by infection or medication with clindamycin and large intestinal GC numbers were not affected by medication in the absence of infection. The recovery of GC in the clindamycin-treated E. pragensis-infected mice may be a direct result of reduced physical damage by the lower numbers of developing parasites. These latter experiments are of practical significance indicating that effective anticoccidial treatment may serve to reduce the susceptibility to opportunistic infection by affecting the numbers of GC retained in the large intestine.

ACKNOWLEDGEMENTS. The authors wish to acknowledge financial support from the Ministry of Education, Science, Sports and Culture, Japan, DEFRA (ALS) and the BBSRC (ALS).

REFERENCES


Fig. 3. The effect of clindamycin treatment on GC numbers in jejunum (A), cecum (B) and colon (C) at day 9 pi in E. pragensis-infected mice. From left to right, columns represent uninfected mice injected with distilled water (DW) [E.p (–) + DW], uninfected mice treated with clindamycin (Clm) [E.p (–) + Clm], infected mice injected with DW [E.p (+) + DW] and infected mice treated with Clm [E.p (+) + Clm]. Each value represents mean and standard deviation of 5 mice. NS, not significant; * p<0.001.