Available online at www.sciencedirect.com
ScienceDirect
Veterinary Parasitology 144 (2007) 208–221
veterinary parasitology
www.elsevier.com/locate/vetpar

Characterization of a *Cryptosporidium muris* infection and reinfection in CF-1 mice

Thomas A. Miller, Frank W. Schaefer III*

U.S. Environmental Protection Agency, 26 West Martin Luther King Drive, Cincinnati, OH 45268, United States

Received 18 August 2005; received in revised form 14 October 2006; accepted 18 October 2006

Abstract

To establish control values for circulating cells and immune associated organs over the course of a self-limiting *Cryptosporidium muris* infection and rechallenge infection, mice were sacrificed at intervals starting before oral inoculation and ending after oocyst shedding had ceased. These values were used in other experiments to evaluate changes in these parameters induced by a single dose glucocorticoid immunosuppression model and in other immunosuppression studies.

Flow cytometry counts of circulating T-lymphocytes and neutrophils, differential leukocyte counts, leukocyte morphology, spleen and thymus changes, and oocyst shedding were evaluated. Immediately after *C. muris* oocyst inoculation and up to the start of oocyst production (day 0 to day 7), the circulating blood profile showed a 50% drop in all leukocytes, including both large and small lymphocytes and CD3, CD4 and CD8 T-lymphocytes. There was an initial slight rise in circulating mature neutrophils after oocyst inoculation but numbers promptly dropped below normal and remaineded below normal. In the differential cell counts, monocytes with a fat, oval morphology increased by 60% at 24 h and remained high through oocyst shedding and beyond (day 8 through day 36). During oocyst shedding and continuing past the end of shedding, T-lymphocytes increased 100%. Monocytes with a flat, angular morphology increased in a similar manner.

Immediately after oocyst inoculation the spleen contracted by 29%, but became 92% larger than its pre-inoculation size by day 14 when heavy oocyst shedding began. It remained enlarged through the end of oocyst shedding (day 29) and beyond (day 36). Spleen volume decreased and increased similar to changes in T-cell numbers. Throughout the *C. muris* infection the thymus remained largely unchanged.

The transit of an oocyst bolus was followed from the stomach through the gut to the colon. No oocysts could be found in the stomach, caecum or feces of mice one half hour after oocyst inoculation. Likewise, an oral bolus of India ink passed from the stomach entirely into the colon after 3 h; therefore, no oocysts from the inoculum passed completely through the intestine and out into the feces.

Recovered mice rechallenged with *C. muris* showed increased B-lymphocyte numbers; however, T-lymphocyte numbers remained level. The large lymphocytes increased after rechallenge, peaking on day 3, then decreased through day 10. B-cell numbers followed a pattern similar to the large lymphocytes. On day 10 of infection monocytes with a fat oval morphology rose sharply while B-cells fell in number.

In both the initial infection and the rechallenge there was no unique blood profile which could definitely indicate a protozoal disease or identify a specific point during the course of the disease. There was no increase in the number of either small or large lymphocytes prior to increases in fat or flat monocytes.
Published by Elsevier B.V.

Keywords: *Cryptosporidium muris*; B-lymphocytes; Reinfection; Leukocytes; CD4; CD8; T-lymphocytes; Thymus; Spleen

* Corresponding author. Tel.: +1 513 569 7222; fax: +1 513 487 2555.
E-mail address: Schaefer.Frank@EPA.GOV (F.W. Schaefer III).

0304-4017/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetpar.2006.10.026

1. Introduction

Cryptosporidium parvum and *C. hominis* are waterborne protozoan parasites of man having public health significance. They cause a transient self-limiting diarrhea in normal immunocompetent adults (Xiao et al., 2004; Tzipori and Griffiths, 1998; Mackenzie et al., 1995; Fayer and Ungar, 1986); however, patients who are immune compromised frequently develop a chronic, debilitating, and often life threatening diarrhea (Gatei et al., 2002; Goodgame et al., 1999; Navin et al., 1999; O'Donoghue, 1995). Studying the host–parasite interaction is complicated by the fact that *C. parvum* (Iowa Isolate) does not naturally infect normal adult immunocompetent mice (Campbell et al., 2002; Taylor et al., 1999; Enriquez and Sterling, 1991; Iseki et al., 1989). It, however, does infect mice whose immune systems are either immunosuppressed chemically (Miller and Schaefer, 2006; Huang et al., 2003; Cifone et al., 1999; Cicmanec and Reasoner, 1997; Cheng et al., 1996), genetically (Mead et al., 1991), or whose immune systems are immature (McDonald et al., 1992; Novak and Sterling, 1991; Rasmussen and Healey, 1992; Scaglia et al., 1991). This means that a direct comparison of the infectivity of *C. parvum* in immunocompetent and immunocompromised mice is not possible; therefore, by using *Cryptosporidium muris*, which naturally infects mice and causes a similar self-limiting disease, such a comparison can be made (Miller et al., 2006).

The major objectives of this study were to characterize the immunologic changes which take place over the entire course of an initial *C. muris* infection from before oocyst inoculation, through and past oocyst shedding, as well as changes after the rechallenge of mice with naturally acquired immunity. This control data was compared with data from immunocompromised mice in infectivity studies (Miller et al., 2006) and for studies which characterized a new glucocorticoid immunosuppression model (Miller and Schaefer, 2006).

The expectation was that the initial inoculation of *C. muris* oocysts would result in an inflammatory immune response characterized by a rise in circulating neutrophils followed later by a somewhat slower rise in lymphocytes. The response to rechallenge was expected to result in an increase in B- and/or T-lymphocytes. A secondary study objective was to identify a unique hematologic profile for a protozoan infection and demonstrate a blood profile for specific points over the course of the infection that would uniquely identify that point in the infection. No unique circulating blood profile emerged for the *C. muris* infection nor for any point in the infection.

2. Methods and materials

2.1. Mice

CF-1 female mice which were 5–7 weeks of age were obtained from Charles River Laboratories (Portage, MI). All mice were housed in groups of 10 and were allowed food and water *ad libitum*. All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) of the U.S. Environmental Protection Agency.

2.2. Cryptosporidium oocysts

C. muris RN 66 strain oocysts were initially obtained from Iseki and Wehl (Osaka City University Medical School, Japan). Oocysts were propagated in 5–7-week-old CF-1 female mice. Ten days after oral inoculation the mice were placed in suspended cages, and the feces were collected daily from day 13 through day 20 post-infection. Oocysts were purified using discontinuous sucrose and cesium chloride gradients (Arrowood and Donaldson, 1996) and were stored in a 0.01% (v/v) Tween-20 solution containing 100 U penicillin, 100 μg streptomycin, and 0.5 μg amphotericin B per ml at 4 °C.

To evaluate the intensity of oocyst shedding over the course of a *C. muris* infection, 4 fecal pellets were collected per mouse and concentrated by zinc sulfate floatation (Ash and Orihel, 1987). One drop from the floatation surface was transferred to a clean slide using a sterile inoculation loop. To each drop, 0.5 μl of Crypt-a-Glo™ (mAb, Waterborne Labs, New Orleans, LA) was added, coverslipped and incubated in the dark for 20 min at room temperature, then examined at 400× using an epifluorescence microscope. The number of fluorescing oocysts counted in 10 high power fields (HPF) were averaged and recorded. All data points were an average of 5 mice.

2.3. Necropsy procedure

Necropsies were preformed as described previously (Miller and Schaefer, 2006). Mice were anesthetized then exsanguinated and blood was processed for differential cell counts and flow cytometry the same day it was drawn.

The spleen was removed, measured, and its total volume was calculated. The size and length of the thymus was approximated in comparison to the heart. A normal thymus consisted of two white lobes which extended from the base of the heart, above the auricles,