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Changes in mouse circulating leukocyte numbers in C57BL/6 mice immunosuppressed with dexamethasone for *Cryptosporidium parvum* oocyst production

Thomas A. Miller, Frank W. Schaefer III*

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

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Abstract

The Iowa strain of *Cryptosporidium parvum* will not propagate in immunocompetent mice, but will successfully infect genetically immunocompromised nude or SCID mice as well as immunocompetent mice which have been immunosuppressed with glucocorticoids. Using dexamethasone–tetracycline is one published method for immunosuppressing mice for the production of *C. parvum* oocysts. However, dexamethasone-induced immunosuppression is variable, because it is dependent on the total daily water consumption of each individual mouse. The changes in circulating leukocytes and other immune system associated organs before, during and after dexamethasone suppression were analyzed for comparison with a new single injection methylprednisolone acetate (MPA) suppression model. The dexamethasone-induced immunocompromised state was associated with a greater than 90% sustained drop in circulating T-lymphocytes, a greater than 700% increase in circulating mature segmented neutrophils and a severe depletion of circulating monocytes. The thymus and spleen decreased in size by over 80%. Oocyst shedding in suppressed mice started within 4 days of oocyst inoculation and persisted for 6 days post-dexamethasone treatment. Seven days after dexamethasone withdrawal, circulating neutrophils still were 549% higher than controls. Circulating CD3 and CD4 lymphocytes remained depressed by 85–90% while on dexamethasone and for 7 days after discontinuing dexamethasone. CD8 lymphocyte numbers initially decreased by 90%, but rose even while on dexamethasone and even with severe thymic involution. At day 7 post-dexamethasone treatment, the spleen was 119 mm^3, approximating the same size as controls. Fourteen days post-dexamethasone treatment, which was 8 days after oocyst shedding had ceased, the CD8 counts per 5000 events were only 1.6% below controls, while the CD3 and CD4 counts were still depressed by 66%. The thymus now was about one quarter smaller than the controls. The rise in circulating CD8 lymphocytes, when oocyst production stopped, suggests that CD8 positive lymphocytes may play a significant role *in vivo* in clearing the parasite. The overall pattern of immunosuppression was nearly identical to that observed with the methylprednisolone acetate immunosuppression model.

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1. Introduction

Cryptosporidium spp. are small protozoan parasites that infect the gastrointestinal tract of a variety of mammals including man (Current and Garcia, 1991; Current and Blagburn, 1990; Fayer and Ungar, 1986). *Cryptosporidium hominis* and *Cryptosporidium parvum*

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

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are of particular interest from a public health perspective with numerous waterborne outbreaks having been reported in both Europe and the United States over the past decade (Xiao et al., 2000; Mac Kenzie et al., 1994). In the immune competent population, cryptosporidiosis is a transient self-limiting diarrheal disease of the small intestine with high morbidity but low mortality. On the other hand, individuals with compromised immune systems are more severely affected, developing a chronic and sometimes fatal parasitism for which there is currently no U.S. Food and Drug Administration approved treatment (Gatei et al., 2002; Crabb, 1998; Goodgame et al., 1993).

Studying the early stages of the host–parasite relationship directly in humans is obviously difficult (Chappell and Okhuysen, 2001), so an alternative host is needed. The most economic laboratory host would be the mouse, but the Iowa strain of *C. parvum* normally does not infect mice (Enriquez and Sterling, 1991). In order to propagate *C. parvum* in normal mice, the resistance to infection must be abrogated or in some way suppressed. Nude (athymic) mice and SCID mice, which have genetic deficiencies in their ability to mount an effective adaptive immune response, become chronically infected upon inoculation (McDonald et al., 1992; Ungar et al., 1990). The athymic mouse lacks T-lymphocytes, while the SCID mouse lacks both T- and B-lymphocytes. Using adoptive transfer of either CD4+ or CD8+ T-lymphocytes back into chronically infected SCID mice, it was determined that CD4+ T-lymphocytes were essential for the eradication of the parasite. CD8+ T-lymphocytes were less critical for recovery but had an effect on the intensity of oocyst shedding (McDonald et al., 1994; Rasmussen and Healey, 1992).

Several methods have been employed to render laboratory mice susceptible to a *C. parvum* infection. One of the more widely used methods of *C. parvum* propagation uses the known effects of cortisone on the cells of the immune system, mainly the apoptosis of lymphocytes, and overall depression of monocytes and eosinophils (Schalm, 1970) to alter the mouse adaptive immune response. Cicmanec and Reasoner (1997) used an alternating oral dexamethasone–tetracycline regimen to achieve immune suppression and to effectively propagate *C. parvum* in C57BL/6 mice. We developed a new, one dose model of immunosuppression using the long acting glucocorticoid, methylprednisolone acetate (MPA) (Miller et al., 2007; Miller and Schaefer, 2006). The purpose of this study was to characterize dexamethasone immunosuppression and recovery and compare this model with the MPA one dose immunosuppression model.

2. Materials and methods

2.1. Mice

C57BL/6 female mice, 5–6 weeks of age, were obtained from Charles River Laboratories (Kingston, NY). The mice were housed in groups of up to10 and were allowed food and water *ad libitum*.

2.2. Experimental design

In accordance with the protocol reported in Cicmanec and Reasoner (1997) C57BL/6 female mice were dosed on alternate days with dexamethasone 21-phosphate then tertracycline hydrochloride *ad libitum* in their drinking water. After completion of the fourth dexamethasone dose, the mice were individually infected with *C. parvum* oocysts. Six days later they were placed in suspended cages and the feces were collected in pans containing reagent grade water. Three mice were selected at random to be sacrificed for organ evaluation and blood collection at each sampling time before, during and after treatment. The data reported was the average of three mice.

2.3. C. parvum source and propagation

The *C. parvum* (Iowa isolate), which was obtained from C. Sterling Parasitology Laboratories (University of Arizona, Tucson, AZ), was originally from Dr. Harley Moon, Ames, Iowa, and maintained by passage through calves. Oocysts were purified at the University of Arizona using discontinuous sucrose and cesium chloride gradients, then stored in a solution containing 0.01% Tween-20, 100 U penicillin and 100 μg gentamicin/ml at 4 °C prior to shipment (Arrowood and Donaldson, 1996).

Suppression and inoculation of the mice followed the procedure outlined by Cicmanec and Reasoner (1997). Briefly, 5–6 weeks old C57BL/6 female mice were treated with alternate day dexamethasone–tetracycline therapy for 8 days prior to oocyst inoculation and maintained on this therapy for 30 days. The dexamethasone (Dexamethasone 21-phosphate, Sigma, St. Louis, MO) was prepared as a 1% (w/v) stock solution in distilled water, then 7.2 ml of the stock solution was added to 250 ml of distilled water and presented as the sole drinking water for the mice. Two hundred and eighty milligrams of tetracycline hydrochloride powder

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