

## Cross-Immunity Between *Hammondia* and *Toxoplasma* Infections in Mice and Hamsters

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Cross-immunity between six strains of *Hammondia hammondi* and the M-7741 strain of *Toxoplasma gondii* was studied in mice and hamsters. Mice and hamsters were inoculated orally with  $10^5$  *H. hammondi* oocysts. All mice and hamsters survived. Four weeks later, they were challenged with 1 to  $10^5$  mean lethal doses ( $LD_{50}$ ) of the *Toxoplasma* oocysts. Animals that died were necropsied. Survivors were killed 30 days after challenge inoculation, and the number of cysts in brain and musculature was determined and compared with that of animals that were not immunized with *H. hammondi*. In one experiment, 36 mice were immunized with each of three *H. hammondi* strains. Four weeks later, the mice were challenged with 1 to  $10^5$   $LD_{50}$  doses of *T. gondii* oocysts. Of 108 mice immunized with *H. hammondi*, 103 survived challenge with *Toxoplasma* oocysts for 30 days, whereas only 3 of 36 unimmunized mice survived with similar doses of *Toxoplasma* oocysts. Fewer *Toxoplasma* cysts were found in mice immunized with *H. hammondi* than in unimmunized mice. In another experiment, groups of six hamsters were each immunized with one of six *H. hammondi* strains and then challenged with  $10^5$   $LD_{50}$  *Toxoplasma* oocysts. All unimmunized hamsters died between 9 and 13 days after inoculation. Percent protection in the various groups of immunized hamsters was: 100, 84, 66, 66, 50, and 33.

*Hammondia hammondi* is a newly described coccidium of cats. It is structurally and antigenically similar to *Toxoplasma gondii* (8, 12). *H. hammondi* is distinguished from *T. gondii* by the fact that tissue cysts but not oocysts are infectious for cats, whereas oocysts are infectious for mice.

*H. hammondi* has an obligatory two-host cycle. The known intermediate hosts (mice, rats, guinea pigs, deer mice, dogs, and hamsters) become infected by ingesting sporulated oocysts of *H. hammondi* that are shed in cat feces; its natural intermediate hosts are not known. *H. hammondi* is of low virulence to mice; it is nonpathogenic for other hosts that have been tested. More is known about its life cycle in the mouse than in any other intermediate host. After the oocysts are ingested by the mouse, sporozoites are released from oocysts and penetrate the intestinal epithelial cells. From 7 to 10 days after oocysts are ingested, tachyzoites multiply in the intestinal lamina propria, muscles, and Peyer's patches, as well as mesenteric lymph nodes, causing necrosis of the infected cells. During the 2nd week of infection, cysts appear in other tissues, primarily in skeletal muscle (5, 8).

Cats become infected by ingesting cysts in the tissues of intermediate hosts. After infected tis-

sue is ingested, bradyzoites are released to initiate the formation of schizonts in the small intestines. Merozoites released from schizonts transform to gametocytes, which in turn produce oocysts in the small intestines. Unsporulated oocysts are shed in feces 5 to 10 days after the ingestion of cysts by the cat. After their sporulation, oocysts are infectious to intermediate hosts, but not to cats. Unlike *Toxoplasma*, *Hammondia* does not infect extraintestinal organs of cats and there is no congenital infection in either definitive or intermediate hosts (3, 5).

*H. hammondi* may be biologically important for two reasons. First, known experimental intermediate hosts (mice, rats, guinea pigs, hamsters, deer mice, and dogs) infected with *H. hammondi* develop antibody against *T. gondii* antigen, which may lead to erroneous diagnosis of toxoplasmosis. Second, hamsters infected with *H. hammondi* resist a fatal challenge with *T. gondii* (8). Because *H. hammondi* is nonpathogenic to nonmurine hosts, it may be useful to immunoprophylaxis of *Toxoplasma* infection in nonfeline hosts (there is no cross-protection in cats). Thus far, cross-immunity between only one strain of *H. hammondi* (CR-4) and a strain of *T. gondii* (M-7741) has been studied (8). In this paper, we report cross-protection between

*T. gondii* and six other strains of *H. hammondi* isolated from the feces of naturally infected cats in the United States.

#### MATERIALS AND METHODS

**Hammondia strains.** Seven *Hammondia* strains were used in this investigation. Strains WC 1170, WC 1984, and WC 2010 were isolated by Wallace (12) from the feces of cats from Hawaii, and they were provided to us by Wallace. The CR-4 strain was isolated from the feces of a cat in Iowa (8). The remaining three strains, C 606, C 656, and C 673, were isolated by us from the feces of naturally infected cats from Columbus, Ohio (2).

**Toxoplasma strain.** The M-7741 strain of *T. gondii* was used for cross-protection studies. Its history has been described (4). Sporulated oocysts of this strain are very pathogenic to mice by the oral route and the mean infective dose (ID<sub>50</sub>) usually equals the mean lethal dose (LD<sub>50</sub>) (4).

**Experimental animals.** Specific-pathogen-free cats were used to obtain *Hammondia* or *Toxoplasma* oocysts. They were raised by their mothers in a closed colony of cats (11). Mice used were of the CFW strain and weighed 20 to 25 g. The golden Syrian hamsters used weighed 100 to 150 g. They were obtained from Midcontinent Research Animals, Shawnee, Kan. All animals were maintained entirely on commercially prepared food.

**Infection of animals.** Oocysts were collected from feline feces after flotation in sucrose solution as described (5). They were sporulated in 2% H<sub>2</sub>SO<sub>4</sub> for 1 week at 22 to 26°C and stored at 4°C. Before oocysts were fed to mice or hamsters, the H<sub>2</sub>SO<sub>4</sub> was neutralized with NaOH (6). Oocysts were counted in the original suspensions. Tenfold dilutions of the oocyst suspension were inoculated orally into mice and hamsters to determine the relation between oocyst numbers and infectivity. At the termination of the experiment, the mice and hamsters were bled to death and then examined for *Hammondia* and *Toxoplasma*. One-half of the unstained brain from selected mice and hamsters was ground in 1 ml of saline, and about a 1/25th portion was examined microscopically under ×100 magnification for cysts. One-square-inch pieces of unstained abdominal muscle were examined for intramuscular cysts. *Hammondia*-infected mouse and hamster tissues were passaged in mice to detect *Toxoplasma* (8).

Tissues for histopathologic examination were fixed in 10% buffered neutral Formalin, and sections were examined after staining with hematoxylin and eosin.

**Serology.** Antibody to *T. gondii* antigen was determined in mouse and hamster sera by the Sabin-Feldman dye test as described (9).

#### RESULTS

All mice and hamsters inoculated with 10<sup>5</sup> *H. hammondi* oocysts survived and developed antibodies to *T. gondii* antigen, in titers of 1:2 to 1:16 for mice and 1:64 to >1:256 for hamsters, 30 days after inoculation. Whereas *H. hammondi* oocysts were not usually lethal to the

hosts, most of the mice and hamsters inoculated with *Toxoplasma* oocysts died of toxoplasmosis (Table 1). Of 31 *T. gondii*-infected mice, 29 died within 14 days. Large numbers of *Toxoplasma* cysts were found in the brains of mice that survived for 30 days; cysts were not seen in abdominal muscles. Of 30 *T. gondii*-infected hamsters, 24 also died of toxoplasmosis. Fewer cysts were found in their brains, and deaths occurred later in hamsters than in mice (Table 1). The ID<sub>50</sub> of *T. gondii* oocysts equaled the LD<sub>50</sub>.

Of 108 mice immunized with *H. hammondi*, 103 survived challenge with 1 to 10<sup>5</sup> *T. gondii* oocysts, whereas few unimmunized mice survived (Table 2). Although cysts were found microscopically mainly in mice challenged with 10<sup>4</sup> and 10<sup>5</sup> *Toxoplasma* oocysts, mice became infected with *Toxoplasma* even by the highest dilution of the oocyst suspension containing 1 viable oocyst, as evidenced by mouse inoculation. Cysts (presumably *Hammondia*) were found in muscles of all mice. The number of cysts in 1 in<sup>2</sup> of abdominal muscle varied between 3 and 28, both in mice infected with *H. hammondi* alone or with *Hammondia* and *Toxoplasma*. Cysts were not demonstrated in the brain of *Hammondia*-infected mice either microscopically or by mouse inoculation.

A variable proportion of hamsters immunized with *Hammondia* died of toxoplasmosis after challenge with 10<sup>5</sup> *Toxoplasma* oocysts (Table 3). All hamsters immunized with the WC 1984 *Hammondia* strain survived, whereas one to

TABLE 1. Mortality in mice and hamsters inoculated with *T. gondii* oocysts

No. of infectious <i>Toxoplasma</i> oocysts fed	Mortality <sup>a</sup>	
	Mice	Hamsters
10 <sup>5</sup>	7.3/N/N	10.7/N/N
10 <sup>4</sup>	8.8/N/N	12.8/N/N
10 <sup>3</sup>	11.1/N/N	16.5/N/N
10 <sup>2</sup>	12.3/N/N	22.0/2 <sup>b</sup> /N
10 <sup>1</sup>	12.8/2 <sup>c</sup> /1	24.5/2/2
10 <sup>0d</sup>	13.0/1/4	N/2/4

<sup>a</sup> Six mice and six hamsters per group. First figure, Mean day of death; second figure, number of animals with chronic *Toxoplasma* infection; third figure, number uninfected. N, No animal in group.

<sup>b</sup> Number of *Toxoplasma* cysts in chronically infected hamsters: 900 and 1,500 in hamsters fed 10<sup>2</sup> oocysts, 400 and 450 in hamsters fed 10<sup>1</sup> oocysts, and 400 and 300 in hamsters fed 10<sup>0</sup> oocysts.

<sup>c</sup> Number of *Toxoplasma* cysts in chronically infected mice: 2,300 and 4,375 in mice fed 10<sup>1</sup> oocysts, and 1,250 in the mouse fed 10<sup>0</sup> oocysts.

<sup>d</sup> 10<sup>-1</sup> dilution was noninfectious to mice and hamsters.

TABLE 2. Mortality in *H. hammondi*-immunized mice after challenge with *Toxoplasma* oocysts<sup>a</sup>

No. of infectious <i>Toxoplasma</i> oocysts fed	Mice immunized with given <i>Hammondia</i> strain								
	C606			C656			C673		
	No. of mice dead <sup>b</sup>	No. of mice with cysts	Mean no. of cysts	No. of mice dead	No. of mice with cysts	Mean no. of cysts	No. of mice dead	No. of mice with cysts	Mean no. of cysts
10 <sup>5</sup>	1 (12)	5	1,005	0	6	127	2 (10,20)	4	168
10 <sup>4</sup>	0	6	540	0	5	446	0	4	243
10 <sup>3</sup>	1 (14)	1	125	0	5	130	0	3	203
10 <sup>2</sup>	0	2	50	0	2	250	0	0	
10 <sup>1</sup>	0	0		0	1	150	0	1	125
10 <sup>0</sup>	0	0		1 (18)	1	375	0	0	

<sup>a</sup> Three groups of 36 mice (total mice, 108) were each immunized with one of three *H. hammondi* strains. Four weeks later, mice were divided in groups of six, and each mouse was challenged with the indicated numbers of *T. gondii* oocysts. All mice were killed 58 days after inoculation with *H. hammondi* oocysts, and the number of cysts was counted in brain and muscles. Brains of mice without demonstrable cysts were inoculated into clean mice for the detection of *Toxoplasma*.

<sup>b</sup> Six mice in each group. Numbers in parentheses indicate day of death for each mouse.

four of six hamsters immunized with each of the other five *Hammondia* strains died of toxoplasmosis (Table 3). *H. hammondi*-immunized hamsters died 1 to 3 weeks later than unimmunized hamsters administered a similar dose (Table 1).

### DISCUSSION

A previous study showed that hamsters immunized with the CR-4 strain of *H. hammondi* survived a challenge with 10<sup>5</sup> *T. gondii* oocysts of the M-7741 strain (8). The present study shows the differences in the immunogenicity of six other *Hammondia* strains. The CR-4 and WC 1984 strains appear to be more immunogenic than the other five strains of *H. hammondi* tested. Finding of viable *Toxoplasma* cysts in the brains of mice and hamsters immunized with *H. hammondi* and challenged with *T. gondii* indicates that *Toxoplasma* is able to multiply in *H. hammondi*-infected animals but at a slower rate than in *H. hammondi*-free mice. The number of *Toxoplasma* cysts in mice or hamsters immunized with *H. hammondi* and in unimmunized animals may be used as another measure of protection afforded by *Hammondia* against *Toxoplasma*. The cysts found in the brains of mice and hamsters immunized with *Hammondia* and challenged with *Toxoplasma* (Tables 2 and 3) are likely to be *Toxoplasma* and not *Hammondia*, because *Hammondia* cysts are rarely detected microscopically in brain, and when they do occur they are much smaller than *Toxoplasma* cysts (8).

The mechanism of immunity between *H. hammondi* and *T. gondii* is not known. To our

TABLE 3. Mortality in *H. hammondi*-immunized hamsters after challenge with *T. gondii*<sup>a</sup>

<i>H. hammondi</i> strain	No. dead	Mean day of death	Mean no. of cysts in brain
WC 1170	2	16.5	200
WC 1984	0	0	91
WC 2010	1	30	130
C606	3	17.3	250
C656	2	18.5	425
C673	4	17.6	300

<sup>a</sup> Thirty-six hamsters in groups of six hamsters were each immunized with one of six *H. hammondi* strains. One month later, they were challenged with 10<sup>5</sup> *T. gondii* oocysts. The survivors were killed on the 30th day after inoculation with *T. gondii*, and the number of cysts was counted in brains. Pooled brains from each group were inoculated into mice for the detection of *Toxoplasma*.

knowledge *H. hammondi* is the only known organism to cross-react antigenically with *T. gondii* in the dye test. Therefore, it is tempting to speculate that *H. hammondi* specifically protects mice and hamsters against *T. gondii*. A number of other antigenically unrelated organisms, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, and *Besnoitia jellisoni*, are known to stimulate nonspecific protection against *T. gondii* infection. However, the degree of protection afforded by these antigenically unrelated organisms can usually be overcome by less than 10 LD<sub>50</sub> doses of *T. gondii*, and in most instances immunization by these organisms merely causes delay in death (7). The degree of protection afforded by *H. hammondi* is undoubtedly supe-

rior to protection by antigenically nonrelated organisms.

*Toxoplasma* is a common cause of abortions in sheep in New Zealand and England (1, 10). The results of the experiments indicate that it would be desirable to investigate the feasibility of immunizing sheep with *H. hammondi* with the object of preventing congenital *Toxoplasma* infection.

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