Enhancement of Humoral Immune Response of Isospora felis-Infected Cats after Inoculation with Toxoplasma gondii

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Toxoplasma gondii (T. gondii), an obligate intracellular protozoan parasite, is prevalent in man and animals throughout the world. Several workers have reported that cats shed transiently a number of oocysts during the acute phase of infection, while chronically infected cats seldom pass out oocysts, even with reinfection [5]. One of the feline coccidia, Isospora felis (I. felis) commonly found in wild and domestic cats is recognized as non-pathogenic. Both parasitic species can penetrate extra-intestinal tissues, and survive for long periods. The relationship between T. gondii and I. felis is biologically and epidemiologically significant, since superinfection with I. felis has been shown to induce reshedding of T. gondii oocysts in cats chronically infected with T. gondii [2, 6]. However, it is still unclear whether such mixed coccidial infections have some influence on the immune response of cats to either I. felis or T. gondii infection.

In the present study, we attempted to examine anti-I. felis antibody in sera of cats infected with I. felis using indirect immunofluorescence assay (IFA). The effect of T. gondii infection on the humoral immune response against I. felis of the cats was likewise studied.

Cysts of S-273 strain of T. gondii were obtained from brains of mice on the 5th to 7th week post-infection and suspended to adjust cyst density to 10⁵ cysts in one ml of saline. Trophozoites of RH strain of T. gondii were prepared from infected mouse embryonal cell culture as described previously [11]. Oocysts of I. felis and T. gondii were isolated from the feces of infected cats by floating in ZnSO₄ solution and suspended to a concentration of 10⁵ oocysts in one ml of saline. Sporulated oocysts of I. felis were isolated by sucrose density gradient centrifugation [1] and excystation was done by incubating the oocysts in phosphate buffered saline (PBS), containing 0.5% taurocholic acid and 0.1% trypsin at 37°C for 30 min. Sporozoites were then isolated by percoll-sucrose density gradient centrifugation [1]. Some of the isolated parasites were fixed in 1.0% paraformaldehyde in PBS at 4°C for 15 min and washed in PBS three times.

Male and female six-week-old cats were kept in individual cages under strict isolation in the Animal Care Facilities at Shionogi-Abara Laboratory, Shiga. Three groups of cats used are as follows; Group 1) one cat that shed no oocysts of either T. gondii or I. felis was inoculated orally with 10⁵ oocysts of T. gondii and on the 46th day post inoculation (p.i.), the cat challenged with 10⁵ oocysts of I. felis (negative control); Group 2) two cats that shed intermittently I. felis oocysts as natural infection were inoculated with 10⁵ of oocysts of T. gondii; and Group 3) consisted of three cats that shed I. felis oocysts as natural infection, two of which were inoculated intramuscularly with 100 mg of freeze-thaw killed T. gondii together with Freund’s complete adjuvant three times at three-weeks interval, and one cat was injected with the same volume of saline instead of killed parasites. On the 1st week post-final injection, they were inoculated with 10⁵ cysts of S-273 strain of T. gondii orally.

For the detection of excretion of oocysts, cats’ feces were collected daily, and examined microscopically [4]. For measuring anti-I. felis and anti-T. gondii antibodies, sera were obtained weekly from each cat from the jugular vein, and stored at -20°C until use. For testing, serum samples were diluted four-fold serially in PBS. Anti-T. gondii IgG antibodies were examined by IFA [11] and their titers are expressed in the text and figures as reciprocal of serum dilution.

![Fig. 1. Aspects of oocyst excretion and antibody production in a cat inoculated with oocysts of T. gondii followed by inoculation with oocysts of I. felis.](image-url)

- A cat which had never been infected with both of T. gondii and I. felis was inoculated with 10⁵ oocysts of T. gondii (S-273 strain), subsequently with 10⁵ oocysts of I. felis on the 46th day p. e. with T. gondii.
- Anti-T. gondii IgG antibody (solid line); anti-I. felis IgG antibody (broken line); T. gondii oocysts per cat per day (dotted line); post-exposure (p.e.)
Anti-*I. felis* IgG antibody titre was determined by IFA following the same procedure used for anti-*T. gondii* IgG determination, using sporozoites instead of *T. gondii* RH strain trophozoites.

Cat of Group 1 shed *T. gondii* oocysts between 20 and 26 days post-exposure (p. e.) with *T. gondii* (Fig. 1). Peak of shedding was on the 21st day p. e. and the total number of oocysts was approximately $1.86 \times 10^7$. Shedding of *I. felis* oocysts was not observed until the end of the experiment, despite inoculation with *I. felis* oocysts on the 46th day p. e. with *T. gondii*.

As shown in Fig. 2, one of the two cats (cat No. 26) in group 2, shed *I. felis* oocysts irregularly from the 9th to the 18th and from the 30th to the 32nd day p. e., and oocyst counts per day ranged from $10^5$ to $10^7$. The other cat (cat No. 18) also shed *I. felis* oocysts transiently from the 4th to the 8th day p. e. prior to inoculation with *T. gondii* oocysts. After challenge with oocysts of *T. gondii*, cats shed *T. gondii* oocysts from the 23rd to 27th day p. e. (cat No. 18), or on the 30th day until the 33rd day p. e. (cat No. 26) respectively. Peak of shedding was on the 24th day (cat No. 18) and on the 31st day (cat No. 26) p. e. The total number of oocysts varied from $0.8 \times 10^7$ (cat No. 18) to $2 \times 10^7$ (cat No. 26).

As shown in Fig. 3a, a cat exposed to cysts showed transient shedding of *I. felis* oocysts on the 21st to the 42nd day before inoculation with *T. gondii* cysts. Thereafter, the cat began to shed *T. gondii* oocysts on the 5th to the 8th day p. e. The total number of oocysts was about $1.2 \times 10^5$. Resheding of *I. felis* oocysts was not observed. In Group 3b, both cats shed *I. felis* oocysts transiently from the 27th to the 41st day before *T. gondii* infection.

Figs. 3a and 3b. Effect of inoculation of *T. gondii* parasites: oocysts excreted and antibody production of *I. felis*-infected cats immunized with either saline (3a) or killed *T. gondii* (3b), and post-challenged with live cysts of S-273 strain of *T. gondii*.

Anti-*T. gondii* IgG antibody (solid lines); anti-*I. felis* IgG antibody (broken lines); oocysts of *I. felis* (thick solid lines) and oocysts of *T. gondii* (dotted lines) per cat per day; post injection of killed *T. gondii* (p. inj.).

Shedding of oocysts of *T. gondii* was observed on the 3rd (Cat No. 21) or the 4th (Cat No. 22) day p. e., and continuously shed for four days. Shedding of *I. felis* oocysts was also detected on the 2nd day before shedding of *T. gondii* oocysts, and continued for four days (Fig. 3b).

Anti-*T. gondii* IgG antibody in Group 1 (Fig. 1) appeared on the 14th day p. e., then increased its IFA titre to 4$^5$, and remained at the same level until the end of the experiment. Anti-*I. felis* IgG antibody was detected on the 28th day to the 35th day p. e., even prior to inoculation of the cat with *I. felis* oocysts. On the 46th day p. e. to *T. gondii*, the cat was inoculated with *I. felis* oocysts. An
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increase of anti-I. felis IgG antibody titre was observed as 4\(^3\) on the 9th day p. e.

In Group 2 (Fig. 2), cats exposed to T. gondii oocysts also showed similar pattern of anti-T. gondii IgG antibody production to that of Group 1. Anti-I. felis IgG antibody also appeared on the 14th day p. e. (cat No. 26) or on the 21st day p.e. (cat No. 18).

Likewise, a cat inoculated with T. gondii cysts showed an increase of anti-T. gondii IgG antibody on the 14th day p. e. and the titre reached to 4\(^3\) on the 28th day p. e. (Fig. 3a). Anti-I. felis IgG antibody peaked to 4\(^3\) on the 21st day p. e., and thereafter gradually decreased to 4\(^1\) on the 77th day p. e.

Cats immunized with killed parasites of T. gondii registered a high level of anti-T. gondii IgG titre from the second injection of killed parasites (Fig. 3b). Anti-I. felis IgG antibody also appeared transiently after the second and third injection of killed T. gondii. After the cats were inoculated with T. gondii cysts, they showed a transient increase of anti-I. felis IgG titre, while anti-T. gondii IgG antibody sustained the high titre level until the end of the experiment.

Our findings demonstrate that in cats infected with I. felis, anti-I. felis IgG antibody titre is seemingly low or non-detectable during the period of oocyst shedding as well as during chronic phase of infection. Several authors have reported that antigenic differences were found between T. gondii sporozoites and trophozoites and the rapid loss of sporozoite-specific antigen was observed after sporozoite invasion into the host tissues [8, 9, 10]. Also, I. felis has been shown to possess the ability to survive in host cells as hypnozoites and induce no host immune reactions [3]. In view of this, our data suggest that antibody-production response to the antigenic stimulation of I. felis sporozoite in cats is apparently low to detect the presence of antibody by IFA. We have no data, however, to show the antigenic differences between I. felis sporozoites and hypnozoites, as well as between I. felis and T. gondii. Thus, further studies are necessary to clarify these questions. Moreover, other assay systems should be developed to detect host immune reactions against I. felis.

Anti-I. felis antibody production was enhanced by exposing the I. felis-infected cats to T. gondii. Interestingly, cats immunized with killed T. gondii also showed the increase of anti-I. felis antibody titre even before inoculated with live T. gondii. This phenomenon may have resulted from the immunopotentiating activity of T. gondii antigens. Our findings seem to agree with studies that documented enhanced anti-tumour and anti-parasite effect(s) of T. gondii and its extracts in animals [7, 12, 13]. Furthermore, cross-immune reaction between T. gondii and I. felis may be another plausible explanation for such a phenomenon. However, studies on the comparative analysis of antigenicity between T. gondii and I. felis are needed.

It is also interesting to note that cats immunized with killed T. gondii did not appreciably suppressed shedding of T. gondii and I. felis oocysts, despite the cats' production of high anti-T. gondii antibody titre. This finding suggests the apparent absence of the effect of humoral antibodies in suppressing oocyst excretion after inoculation with T. gondii cysts. A better understanding of this phenomenon warrants further studies related to mechanism of local immune reaction(s) and cell-mediated immunity involving intestinal parasitism.

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REFERENCES