Interleukin-12, Interferon-γ and Interleukin-4 Gene Expression in Cats Infected with Toxoplasma gondii

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ABSTRACT. Interleukin-12, Interferon-γ and Interleukin-4 mRNA levels in cells of the spleen and mesenteric lymph nodes of cats following primary and secondary infection with Toxoplasma gondii were examined by a semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) method. Expression of Interleukin-12p40 mRNA and Interferon-γ mRNA was observed after primary and secondary oral infection with Toxoplasma gondii. In contrast, no expression of IL-4 mRNA in the spleen and little expression in the mesenteric lymph nodes were observed after primary infection when the cats shed oocysts, however, the expression of IL-4 mRNA was observed in the cats after secondary inoculation.—KEY WORDS: cytokine, feline, Toxoplasma gondii.


Toxoplasma gondii (T. gondii), the intracellular parasite, causes congenital abnormalities in humans and animals which are first evident during pregnancy. In immunocompromised individuals, this parasite causes intense encephalitis. Felids, the definitive host of T. gondii, shed environmentally resistant oocysts in feces after primary infection, therefore, feline toxoplasmosis is one source of transmission of the disease. After primary infection, the infected cats show no oocyst shedding upon reinfection, except in cases involving an immunosuppressive condition, or co-infection with feline coccidiosis [2, 3]. Protective immunity to T. gondii in murine toxoplasmosis is mediated mainly by cellular immune responses. Interferon-γ(IFN-γ) produced by CD4+ and CD8+ T-cells, natural killer (NK) cells and activated macrophages induces a microbicidal state which is involved in resolution of the infection for the intracellular parasites [5, 21]. Recent studies show that interleukin-12 (IL-12), a heterodimeric cytokine, is essential for stimulation of NK cells, differentiation of Th1 cells and induction of IFN-γ production. These enhances protection against T. gondii infection [5–7, 17, 19], and production of IL-12p40 (inducible subunit) is regulated by interleukin-4 (IL-4) and/ or IFN-γ that results regulation of two helper T cell subsets (Th1, Th2). Although the immune responses in feline toxoplasmosis have been investigated [8–11], the role of such cytokines in prevention of oocyst shedding is still unknown. In the present study, we examined the levels of IL-12, IFN-γ and IL-4 mRNA in cats infected with T. gondii using semi quantitative Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) to determine whether expression of these cytokines is regulated in response to infection.

Cysts of T. gondii (Beverley strain) were obtained from brains of mice on the 35th day post-inoculation and were suspended in Dulbecco’s modified Eagle medium (D-MEM) (GIBCO BRL, Grand Island, NY) at 10^5 cysts/ml. Three-month-old male and female Specific Pathogen Free cats were supplied by Aburahi Laboratories of Shionogi Co., Ltd. They were kept in individual isolated cages and fed only dry chow throughout the experiments. All cats were inoculated orally with approximately 200 cysts per day from 5 days to 8 days post inoculation. Seven days after the first inoculation, three cats were sacrificed for isolation of T. gondii enteroepithelial-stage parasites and many enteroepithelial-stage parasites were observed in their intestinal tracts. Also, three months post-inoculation, one cat was sacrificed and the remaining three infected cats were reinoculated orally with the same number of cysts and sacrificed on the 1, 2 and 3 days post reinoculation. The spleens and mesenteric lymph nodes were obtained from these 7 cats. One hundred micrograms of total RNA was extracted from approximately 0.1 gram of the tissues in 1 ml of TRIzol® (GIBCO BRL, Grand Island, NY), and approximately 5 µg of messenger RNA was isolated using the BioMag mRNA purification kit® (Perseptive Diagnosis, Framingham, MA). This mRNA was employed as the template for the first strand cDNA synthesis using a random primer (5 ‘-pd (NNNNNN)-3’; Takara, Ohtsu, Japan) and Super Script II reverse transcriptase (GIBCO BRL, Grand Island, NY). RT-PCR amplification was performed using a 100 µl reaction mixture (50 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.1% Triton X-100, 2 mM MgCl2; 0.4 mM of each dNTP, 50 pmol each of the sense and anti-sense primers) containing 2.6 units of Taq DNA polymerase (Boehringer Mannheim, Tokyo, Japan). The sequences of the primers employed have been described elsewhere [HPRT-20, IL-4:14], or were designed based on other mammalian cytokine mRNA sequences [GenBank accession No. D30619 (feline IFN-γ), M65290 (human IL-12p40)] (Table 1). The PCR conditions employed were 94°C for 60 sec, 50°C for 90 sec,
72°C for 120 sec for each of the cycles as determined experimentally (Table 1, Fig. 1). To standardize the concentration of input cDNA, a fragment of feline hypoxanthine-guanine phosphoribosyl transferase (HPRT) mRNA was cloned and sequenced as a housekeeping gene (deposited in DDBJ and GenBank under accession No. AB005557). The RT-PCR amplifications were performed three times and identical results were obtained in each instance. Contamination of genomic DNA did not occurred because the sequences has introns and PCR amplification of 0.2 µg feline genomic DNA under the same conditions did not produce same bands. Levels of expression of IL-4 mRNA, IL-12p40 mRNA and IFN-γ mRNA in cells of the spleen and mesenteric lymph nodes of cats infected with T. gondii were measured by semi-quantitative RT-PCR [14, 20]. The amounts of each of the PCR products obtained with varied concentrations of input cDNA increased dose dependently (Fig. 1). The RT-PCR reaction products were analyzed by video densitometry (ATTO Densitograph software library Version 4.0: Tokyo, Japan) and for each type of cytokine examined the ratio of cytokine mRNA:HPRT mRNA was calculated and plotted (Fig. 2). Amplified DNAs were inserted into a plasmid vector (pMos blue T-vector: Amersham Life Sciences, Buckinghamshire, England) and DNA sequencing of both strands was performed for each of five independent clones by the dideoxy chain termination method (Abi PRISM Dye terminator sequencing kit: Perkin Elmer, Foster City, CA). The sequences of the PCR products showed 100% homology when compared with the sequences of IL-4 mRNA [15], IFN-γ mRNA [1] and IL-12p40 mRNA [16].

An expression of IL-12p40 subunit mRNA and IFN-γ mRNA in cells of the spleen and mesenteric lymph nodes of cats was observed after both primary and secondary inoculation with T. gondii (Fig. 2). In the cats orally inoculated with cysts of T. gondii, the parasites penetrated the tissue organs from the intestinal tract within a few hours. Generally, IFN-γ production in an early phase response of inflammation is recognized as non-specific reaction, while Th1 cells produce IFN-γ specifically as a response mediated by an antigen-recognition process. Subauste et al. [18] reported that human related V γ9+V δ2+γδ T cells were cytotoxic for T. gondii-infected cells in an MHC-unrestrected manner, and produced IFN-γ, IL-2, TNF-α, but not IL-4 when incubated with cells infected with the parasite. Based on these findings, it seems that the mechanism of production of IFN-γ in some cats after primary infection may be different from that in cats after secondary infection.

In one of three cats sacrificed on the 7th day after primary inoculation, no marked expression of either IL-12p40 mRNA and IFN-γ mRNA was observed in the spleen, however, expression of both was detected in the mesenteric lymph nodes (Fig. 2). It is unknown to explain this phenomenon in the present study. In order to clarify this, it is necessary to examine the existence of the parasites and production of other cytokines in the spleen of the cat.

No expression of IL-4 mRNA in the spleen and little expression in the mesenteric lymph nodes were observed after primary infection when the cats shed oocysts. However, the expression of IL-4 mRNA was observed in the cats after secondary inoculation (Fig. 2). These findings suggest that the expression of IL-4, which takes part in

<table>
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<th>Name</th>
<th>[Reference]</th>
<th>Sense primer (5’–3’)</th>
<th>Anti-sense primer (5’–3’)</th>
<th>Predicted PCR product (seq)</th>
<th>No. of cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRPT</td>
<td>[19]</td>
<td>gttggatacaggccagacttgttg</td>
<td>gattcaactgctcacttaggc</td>
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<td>30 cycle</td>
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<td>tattaatgggtctcttacaacc</td>
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<td>397 bp (Human) 343 bp (Bovine)</td>
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<td>[Cat:D30619a]</td>
<td>aattgtctccttactacgaa</td>
<td>attatttcagatgctctacgg</td>
<td>295 bp (Cat)</td>
<td>35 cycle</td>
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<tr>
<td>IL-12p40</td>
<td>[Human:M65290]</td>
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<td>actcctgttggccctctgtga</td>
<td>517 bp (Human)</td>
<td>35 cycle</td>
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Fig. 1. Video densitometric analysis of RT-PCR product signals for cytokine cDNA and housekeeping gene cDNA as a function of the amount of input cDNA. Each of the cDNAs was serially diluted. Ten µg of each RT-PCR product was electrophoresed in 1.5% agarose gel and the density of the bands was analyzed by video densitometry. Each density value was normalized to the value obtained with the maximum amount of cDNA amplified, 0.2 µg.

Table 1. Sequences of the primers employed in amplification of cytokine mRNA by semi-quantitative RT-PCR
induction of humoral immunity, enhances production of specific antibodies against the parasites. This is consistent with the fact that cats infected with T. gondii produce anti-T. gondii IgA and IgG antibodies more than seven days after primary inoculation [12] and secretary IgA antibody specific for T. gondii exists in the intestinal tract more than three weeks after primary inoculation with T. gondii [13].

It is known that cellular immunity related to the expression of IL-12 and IFN-γ is induced in mice after T. gondii infection and those responses involving cellular immunity play a protective role against the parasites [4, 7, 17]. Our findings suggest that, in cats, expression of IL-12 and IFN-γ may be induced by the parasites following infection with T. gondii when the parasites invade the lymph nodes and spleen in the host.

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REFERENCES