NOTE *Parasitology*

Comparison of the Growth Inhibitory Effects of Canine IFN-α, -β and -γ on Canine Cells Infected with *Neospora caninum* Tachyzoites

Yoshifumi NISHIKAWA[1,2], Akira IWATA[3], Hideyuki NAGASAWA[1], Kozo FUJISAKI[1], Haruki OTSUKA[2] and Takeshi MIKAMI[1]

[1]*National Research Center for Protozoan Diseases, Obihiro University, Inadacho, Obihiro, Hokkaido 080–8555,* [2]*Department of Global Agricultural Science, The University of Tokyo, Yayoi 1–1–1, Bunkyo-ku, Tokyo 113–0032 and* [3]*Nippon Institute for Biological Science, 9–2221–1 Shin-machi, Ome, Tokyo 198–0024, Japan*

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ABSTRACT. The growth inhibitory effects of recombinant canine interferon alpha (IFN-α), beta (IFN-β) and gamma (IFN-γ) were examined on Madin-Darby canine kidney cells infected with *Neospora caninum* tachyzoites. The parasite growth was inhibited by all IFNs in a dose-dependent manner. IFN-γ inhibited the parasite growth with greater efficacy than IFN-α or IFN-β. Moreover, the effect of IFNs on *N. caninum* growth associated with the suppression of the host cell viability. The present study indicates IFN-α and -β, besides IFN-γ, play a crucial role for *N. caninum* growth in host cells.

KEY WORDS: canine cell, interferon, Neospora caninum.

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Neospora caninum is a recently recognized protozoan parasite, which was reported in 1988 for the first time [4]. This parasite affects a wide range of mammalian species and is distributed worldwide. Infection with *N. caninum* causes paralysis and death in young livestock and companion animals [5]. The life cycle of the parasite was considered to be one of the coccidian species when its oocyst was found in dog faeces [13]. Thus, dogs can serve both as definitive and intermediate hosts. *N. caninum* infection associated with abortions and stillbirth in cattle, and neurologic disease in calves makes neosporosis an important economic and agricultural concern [6].

Interferons (IFNs) are characterized primarily by their antiviral activity [7]. Due to their multifunctional nature, such as antiproliferative and immunomodulatory effects, IFNs have been used in clinical application against a number of diseases [3]. The IFNs are divided into types I and II. Interferon-α, -β, -τ and -ω are a member of the type I IFN, whereas IFN-γ is that of the type II IFN.

Ovine and bovine IFN-γ suppressed the intracellular multiplication of *N. caninum* in fibroblasts and brain cells, respectively [9, 21]. Also, mouse IFN-γ inhibited the parasite growth in macrophages by a nitric oxide (NO) mechanism [20]. IFN-γ knockout mice and mice treated with antibody against IFN-γ were highly susceptible to *N. caninum* infection [12, 19]. Thus, IFN-γ has been well studied in the context of parasite growth and host defense against parasite infection. However, it is unknown effects of IFN-α and -β on *N. caninum* growth in host cells. In this study, we compared the effects of IFN-α, -β and -γ on *N. caninum* growth in host cells.

N. caninum tachyzoites of the Nc-1 strain [4] were maintained in human foreskin fibroblast cells (Hs68) cultured in DMEM (SIGMA, MO, U.S.A.) supplemented with 10% heat-inactivated fetal bovine serum (FBS). For the purification of tachyzoites, the parasites and host cell debris were washed twice in cold phosphate-buffered saline (PBS) and the final pellet was resuspended in cold PBS and passed through a 27-gauge needle and a 5.0-μm-pore filter (MILLIPORE, MA, U.S.A.).

The *Autographa californica* nuclear polyhedrosis virus and its recombinant viruses were grown in *Spodoptera frugiperda* (Sf9) cells in a TC-100 insect medium (GIBCO BRL, MD, U.S.A.) supplemented with 10% FBS and 0.26% Bacto tryptose broth (Difco, MI, U.S.A.).

The cloning of canine IFN-α, -β and -γ was performed by reverse transcriptase-polymerase chain reaction (RT-PCR) using a pair of the primers, 5-GGC CCC ATG GCC CTG CCC TGC TCC TTC TCG-3' and GCG CGA ATT CTC ATT TCC TCC TCC TGA TTC (for IFN-α), 5'-CCG AAT TCA TCG AGA TGG TAA TAG GTG A-3' and 5'-CAG TCG ACT CAG TTC TGG AGA TAA TCT G-3' (for IFN-β), and 5'-CCG GAT CCA TGA ATT ATA CAA GCT ATA TCT-3' and 5'-CCG CAT CCT TAT TTC GAT GCT CTG CGG CCT-3' (for IFN-γ). Sf9 cells were co-transfected with a transfer vector, pBac-PAKIS (CLONTECH, CA, U.S.A.), in which canine IFN-α, -β or -γ cDNA were cloned, and BaculoGold® Baculovirus DNA (PharMingen, CA, U.S.A.) using Lipofectin™ reagent (GIBCO BRL). After 4 days of incubation at 27°C, the culture supernatant containing recombinant viruses was harvested and subjected to plaque purification. After three cycles of purification, recombinant viruses expressing canine IFN-α, -β or -γ were obtained.

The antiviral activities of canine IFN-α, -β and -γ were assayed by its ability to inhibit cytopathic effects of vesicular stomatitis virus grown in A72 cells in 96-well tissue culture plates, and were shown as a laboratory unit (LU) [10, 11]. The supernatants were centrifuged at 100,000 × g for 120 min to remove viral particles and checked the absence of infectious virus by plaque assay.

Madin-Darby canine kidney (MDCK) cells (5×10^5)

were cultured with *N. caninum* tachyzoites (2×10^6). After incubation for 4 h at 37°C, the cells were washed with PBS and then the infected-cells were cultured with various concentrations of recombinant canine IFN-α, -β or -γ. To quantify the parasite growth, the number of tachyzoites was counted at the end of cultivation (24, 48 and 72 hr-post infection). The cells were scraped and then passed through a 27-gauge needle. The tachyzoite numbers were counted by a hemocytometer.

MDCK cells were plated into 96-well microplates at 1×10^5/200 μl/well, and were then cultured with recombinant IFNs and *N. caninum* tachyzoites (4×10^5). After incubation for 20 hr at 37°C, 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt, and 1-methoxy-5-methylphenazinium methylsulfate (Cell Counting Kit-8, Dojin Laboratories, Japan) were added to final concentrations of 5 mM and 0.2 mM, respectively, and further incubated for 1 hr. The absorbance of the supernatant was measured at 450 nm using a MTP-120 micro plate reader (CORONA ELECTRIC, Japan). The host cell viability (%) is expressed as [(the absorbance of cells treated with parasites and/or IFN) / (the absorbance of cells cultured with medium alone) × 100].

The number of tachyzoites grown in MDCK cells containing 100 LU/m*l* IFNs was lower than that in the control group at 48 and 72 hr after infection (Fig. 1). The inhibitory effect of IFN-γ on parasite growth was highest among three IFNs though there was no significant difference between IFN-α and -β effects. The number of tachyzoites did not increase in MDCK cells containing IFN-γ. Moreover, the treatment with IFNs inhibited the tachyzoite growth in a dose-dependent manner (Fig. 2). MDCK cells were suitable for tachyzoite growth by IFN-γ since the growth was decreased 1/4 in the presence of 1 LU/m*l* IFN-γ.

The host cell viability in the presence of three IFNs was compared (Fig. 3). The host cell viability reduced following *N. caninum* infection in the presence of IFN-γ as compared with IFN-α and -β. The host cell viabilities without *N. caninum* infection were higher than those with the infection.

The present study indicated that canine IFN-α and -β suppressed the intracellular multiplication of *N. caninum* in MDCK cells. In addition, canine IFN-γ inhibited the parasite growth more effectively than canine IFN-α and -β. In *Toxoplasma gondii*, pretreatment of human retinal pigment epithelial cells with recombinant human IFN-α, -β and -γ inhibited the parasite replication [15]. Of these IFNs, IFN-γ was most potent and completely inhibited *T. gondii* replication, which seems to agree with the present finding. Human IFN-γ was shown to have a leishmaniacidal effect greater than human IFN-β on *Leishmania tropica major* amastigotes in human monocytes [17]. Also, mouse IFN-γ was more effective than mouse IFN-α and -β on trypanocidal activity against *Trypanosoma cruzi* in mouse 3T3 fibroblasts and J774 macrophage-like tumor cells [18]. Thus, IFN-γ is considered to be a most important factor on parasite growth. The present data indicated that IFN-γ played a crucial role in *N. caninum* growth.

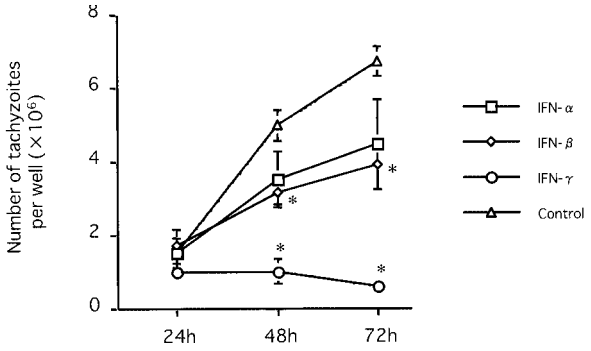

Fig. 1. Effect of IFNs on tachyzoite growth in time courses. MDCK cells (5×10^5) were infected with 2×10^6 of parasites in the presence of canine IFNs (100 LU/m*l*). After 24, 48 and 72 hr, the number of parasites was counted. Results are expressed as the mean of parasite number ± standard deviation of triplicate. (*) According to Student's *t*-test, the differences between control and IFN treatment were significant at the same end of cultivation ($P<0.01$).

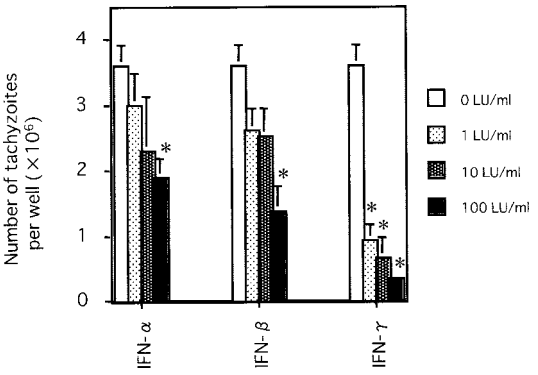

Fig. 2. Effect of IFNs on tachyzoite growth in a dose-dependent manner. MDCK cells (5×10^5) were infected with 2×10^6 of parasites in the presence of serially doses of canine IFNs. After 72 hr, the number of parasites was counted. Results are expressed as the mean of parasite number ± standard deviation of triplicate. (*) According to Student's *t*-test, the differences between control and IFN treatment were significant ($P<0.01$).

The experiment using different strains of inbred mice infected with *Trypanosoma brucei rhodesiense* showed that IFN-α and -β, besides IFN-γ, were induced resistance in mice against the parasite infection [2]. Moreover, IFN-β protected mice against a lethal infection with *T. gondii* [16]. These reports showed IFN-α and -β were other important factors for protective immunity against parasite infection. The present studies showed that IFN-α or -β may also play a crucial role for host immune responses to *N. caninum* infection.

IFN-dependent growth inhibition might be mediated by IFN-specific receptors on the parasite-infected cells. The inhibition of *T. gondii* growth is induced through NO-medi-

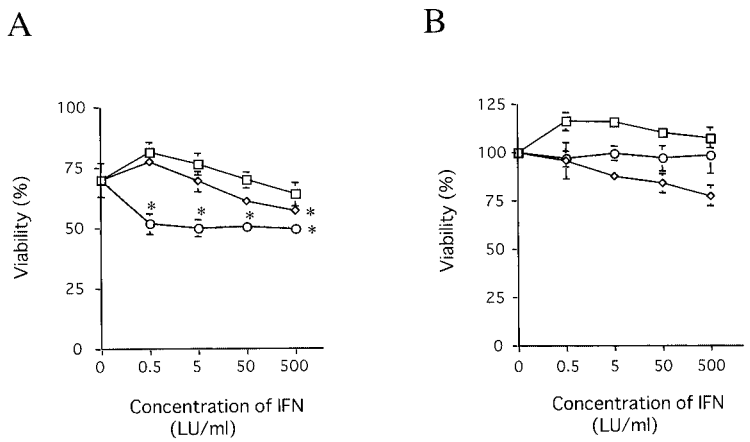

Fig. 3. Effect of IFNs to host cell viability. 1×10^5 of MDCK cells were infected with 4×10^5 of parasites in the presence of serially doses of IFN. After 20 hr, host cell viability was measured and expressed as mean of viability ± standard deviation of triplicate. (A) *N. caninum*-infected MDCK cells. (B) Uninfected MDCK cells. (□) Treatment with canine IFN-α. (◇) Treatment with canine IFN-β. (○) Treatment with canine IFN-γ. (*) According to Student's *t*-test, the differences between the absence of IFN and the presence of various concentration of IFN were significant ($P<0.05$).

ated mechanisms [1, 8], tryptophan depletion [15] or toxic oxygen metabolited [14]. In *N. caninum* infection, IFN-γ suppressed the growth in macrophages by NO mechanism [20]. Although the mechanisms induced by IFN-α and -β are well unknown, indoleamine 2,3-dioxygenase-dependent mechanisms might have a crucial role for control the growth of *N. caninum* as the case of *T. gondii* [15]. In the present study, the effects of canine IFN on *N. caninum* growth associated with the suppression of the host cell viability, suggesting that the viability might affect parasite growth. This data demonstrates, for the first time, the effect of canine IFNs on growth of *N. caninum* tachyzoites. Future studies will focus on the exact mechanisms of IFNs in the inhibitory effects on *N. caninum* growth.

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