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# Cytokine Production in *Hymenolepis Nana* Infection

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## ABSTRACT

**Background:** Although many experimental studies provide convincing evidence that type II immunity is protective against helminths, recent data in mice demonstrate that Th1 is also important in some cestodes like *Hymenolepis nana*. **Objective:** To identify the role of Th1 and Th2 lymphocytes in immunity against *H. nana*, the levels of IL-12, IFN $\gamma$ , IL-5, and IL-13 were determined in serum of humans infected with this cestode. **Methods:** A total of 31 patients (case) with *H. nana* infection and 30 clinically healthy individuals (control) was included in this study. Measurements of IL-12, IFN $\gamma$ , IL-13 and IL-5 in serum samples were performed by solid-phase sandwich enzyme linked immunosorbant assay. Differential leukocyte count was also done. T test, Mann Whitney U test and Wilcoxon W test were used for data analysis. **Results:** The mean concentrations of IFN $\gamma$ , IL-12 and IL-5 in the sera of patients with *H. nana* infection were higher than the control group, but only the differences between the concentrations of IFN $\gamma$  ( $p < 0.001$ ) and IL-13 ( $p < 0.05$ ) in the two groups were significant. There was an increase in the percentage of monocytes, eosinophils and lymphocytes in patients when compared to the controls, but this increase was not significant. **Conclusion:** Results from the present study in humans are in agreement with experimental studies in animals in which both Th1 and Th2 responses occur in *H. nana* infection.

**Keywords:** *Hymenolepis nana*, Cytokines, Th1 and Th2

## INTRODUCTION

The mechanism of parasite survival in the immunized host has been the most interesting unsolved problem in immunoparasitological investigations (1). T cells mediate many responses relevant to immunity against parasitic infections. Many experimental studies have been performed on the role of T helper (Th) subsets and the cytokines that they release (2). Th cells can be classified on the basis of cytokine production: Th1 cells release IL-2, IFN $\gamma$  and lymphotoxin and Th2 cells produce IL-4, IL-5, IL-9, IL-10 and 13 (3). An overwhelming majority of data indicate that type-2 immunity (Th2 cells) has the major role in mammalian protection against helminth infections. IL-4 is important for the induction of IgE. IL-5 and IL-9 act via eosinophil and mast cell stimulation.

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It has been suggested that IL-13 acts via secondary induction of TNF, but its definitive mechanism remains obscure (2-4).

Although many studies provide convincing evidence that type II immunity is protective against helminths, recent experimental studies in mice reveal that Th1 (IFN $\gamma$ , IL-2) are also important in some cestodes like *H. nana* and *H. diminuta*, especially in some stages of infection (4-8).

The immunoprotective function of cytokines produced during *H. nana* egg infection in mice was examined by Asano (5). The results showed that IFN $\gamma$  is a more important or a more essential cytokine in producing immunity to *H. nana* reinfection than IFN $\alpha$  or IL-1 beta. Toenjes examined the cytokines produced by peritoneal cells, splenocytes and mesenteric lymphnode cells during the first week of infection with the larvae of *Taenia crassiceps* in BALB/c mice. Initial immune response was mediated by an increase in IFN $\gamma$  which was quickly followed by an increase in IL-10 production and subsequent reduction in the amount of IFN $\gamma$  (6). Analysis of cytokine production (IFN $\gamma$ , IL-2, IL-3, IL-4 and IL-5) by in vitro Con A stimulated mesenteric lymphnode cells, measured daily after egg or cyst infection of mice with *H. nana* showed that cytokine production varies during parasite development and between different host strains such as BALB/c and C3H/He mice (7). *H. nana* specific clonal lymphocytes were generated from mesenteric lymph nodes of BALB/c mice infected with *H. nana*. Some of their functions were analyzed in vivo and in vitro. The results suggested that helper T cells, especially the Th1 subtype, is involved in protective immunity against *H. nana* (8).

All of the above studies were performed in experimental animals and there is no study done on defense mechanisms against *H. nana* infection in humans. In the present investigation, the level of IL-12, IFN $\gamma$ , IL-5 and IL-13 cytokines in serum of *H. nana* infected humans were determined to demonstrate the role of Th1 and Th2 subtypes in immunity against *H.nana*.

## MATERIALS AND METHODS

**Patients.** Thirty one patients (17 males and 14 females) with *H. nana* infection were included in this study. All were immunocompetent. They did not receive any medication. The diagnosis of *H. nana* infection was based on the detection of *H. nana* egg in stool examination (flotation method). Patients with mixed parasite infection or with signs of chronic, viral and bacterial infections (fever, CRP positive) were excluded.

**Control Group.** 30 clinically healthy individuals without inflammatory or autoimmune diseases matched with patients by age ( $\pm 5$  years) and gender were included as control subjects. In clinical exam initiation they were healthy, without signs or symptoms of any disease. They did not receive any medication and stool examination for OP and OB was negative. CRP test was also negative.

**Sample Collection.** Five milliliters of peripheral blood were withdrawn from each individual after obtaining informed consent. This study was approved by the Ethics Committee of Mazandaran Medical Sciences University. Samples were divided into 2 aliquots; one aliquot was mixed with an anticoagulant (EDTA 5%) for blood cell counting (CBC) and the other aliquot was coagulated for serum separation. Serum was obtained by centrifugation at 1600 xg for 15 minutes. Serum samples were stored at -70°C until analyzed.

**Cytokine Assays and CBC.** Cytokines were detected using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits with paired cytokine-specific monoclonal antibodies according to the manufacturer's recommended procedure ((Bender Med Systems; Vienna, Austria). The detection limit for IL-5, IL-10, IL-12 and IFN- $\gamma$  were 1.46, 0.99, 2.1, and 0.99pg/ml, respectively. CBC was done by Coulter T 890 E (USA) and then differentiation of leukocytes (Neutrophils, Basophils, Eosinophils, Monocytes and Lymphocytes) was performed.

**Statistical analysis.** The results are presented as mean  $\pm$  standard deviation (SD). T test and Mann Whitney U test were used to compare the mean of cytokine concentrations in the case and the control groups. Mann Whitney U and Wilcoxon W tests were used for the analysis of leukocyte subpopulations in the two groups. The correlation between the levels of different cytokines in patients was established by the Pearson test. In all cases  $p < 0.05$  was considered as significant. Results were analyzed with SPSS software.

## RESULTS

A total of 31 patients with *H. nana* infection and 30 healthy individuals as control group were studied. General characteristics of the subjects are summarized in Table 1.

**Table1. General characteristics of case and control groups**

Groups	Gender		Age (X $\pm$ SD) (Years)
	Male (No)	Female (No)	
Case	17	14	32.9 $\pm$ 13.9
control	15	15	35.1 $\pm$ 15.2

-SD:standard deviation

The mean concentrations of IFN $\gamma$ , IL-13, IL-12, IL-5 in the sera of patients with *H. nana* infection (cases) were higher than the control group, but only the differences between the concentrations of IFN $\gamma$  ( $p < 0.001$ ) and IL-13 ( $p < 0.05$ ) in the two groups were significant (Table 2).

**Table 2. Comparison of serum levels (mean  $\pm$  SD) of the various cytokines in case and control groups**

cytokines	Groups		statistical test	Pv
	Control (pg/ml)	Case (pg/ml)		
IFN $\gamma$	3.8 $\pm$ 4.3	9.6 $\pm$ 6.3	4.2	0.0
IL-13	2.6 $\pm$ 3.6	12.4 $\pm$ 25.9	2.0	0.044
IL-12	7.2 $\pm$ 12.5	8.3 $\pm$ 14.3	0.3	0.46
IL-5	8.9 $\pm$ 11.07	15.5 $\pm$ 16.0	1.8	0.06

Correlation between cytokine levels in patients (cases) was also studied and no correlation was found.

To investigate the leukocytes and its subpopulation response in *H.nana* infection, total leukocyte count/ml of blood and percentage of each subpopulation were also determined. There was an increase in percentage of monocytes, eosinophils, lymphocytes

and a decrease in neutrophil percentage in patients compared with the control group but such differences were not significant.

## DISCUSSION

The present study demonstrated that both Th1 and Th2 cytokines increase in serum of humans after *H. nana* infection. This increase was significant for IFN $\gamma$  ( $p < 0.001$ ) and IL-13 ( $p < 0.05$ ). In an experimental study, Conchedda (7) showed that cytokine production varied during infection from one stage of development to another and between different strains of mice (BALB/c and C3H/He). Their data indicated that egg infection stimulate a rapid increase in IFN $\gamma$ , independent of mouse strain. But only in BALB/c mice, IL4 and IL5 were stimulated after 4-5 days post infection, when the parasites are thought to begin their luminal phase. They concluded that there is a predominant Th1-type response during the tissue phase of *H. nana* development and a Th2 polarization occurs during the first few days of the luminal phase. They suggest that egg penetration even during the phase of autoinfection is the major stimulus for Th1 responses. Our data confirm that both Th1 and Th2 mediate responses that have a direct role in immunity to *H. nana*, but since our study was not experimental and autoinfection is possible when the infection is patent, we can not confirm in which stage of infection does polarization of Th1 and Th2 occur. However, it is obvious that in *H. nana* infection, the polarized response of Th1 is elicited during infection by the development of the post oncospherical stages in the intestinal villi and a Th2 polarization appears by the stages in which *H. nana* develop in the lumen (10). As meta-zoan pathogens, parasitic helminths cannot be eliminated by the phagocytic activity of macrophages and neutrophils, rather the typical anti-helminth response is dominated by antibody, complement and the release of tissue-damaging molecules (e.g. eosinophil cationic protein) aimed at destroying the helminth's outer surface and organs of attachment. This response is governed, in large part, by CD4<sup>+</sup> Th2 cells (11).

IL-12 must be secreted by antigen presenting cells during the initial infection in order to develop Th1 polarization (9), but autoinfection in *H.nana* can cause continuous infection and increase the level of IL-12 along with Th1 and Th2 cytokines.

IFN $\gamma$  is one of the representative Th1 cytokines involved in the clearance of intra cellular pathogens. This cytokine regulates antibody production, increases MHC class II and activates macrophages to produce TNF and free oxygen radicals. From studies concerning experimental depletion of macrophages in BALB/c mice, it was suggested that these cells can act in protective immunity to *H. nana* challenge (8).

Although the requirement for type 2 cytokines in worm expulsion is well established, the immune effector cells and molecular mechanisms elicited by this type of immune response have remained elusive. In the case of *T. spiralis*, clear evidence exists that mast cells can play an important role in worm expulsion. However, expulsion of a number of intestinal nematode parasites—while dependent on type 2 cytokines—can occur in the absence of classical effector mechanisms associated with type 2 responses such as B cell activation, antibody production, eosinophilia and mastocytosis (13).

Differential leukocyte count indicated a nonsignificant increase ( $p > 0.05$ ) in eosinophils, lymphocytes and monocytes, but a decrease in neutrophils. The increase may be due to chronic inflammation as a result of *H. nana* infection (12).

Results from the present study are in agreement with the experimental studies in which both Th1 and Th2 responses occur. Th1 responses were directed against the oncosphere

and tissue phase and Th2 responses were against the luminal phase. In our work, the cytokine concentrations were measured in serum and due to the low concentration of cytokines in serum, further work is needed to evaluate the level of these cytokines in lymphocyte secretions after their in vitro stimulation with *H. nana* antigen.

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