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Production of interferon-γ and interleukin-4 by splenocytes in mice infected with *Paragonimus westermani*

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Abstract: The TH cytokine responses of spleen cells stimulated with Con A from mice infected with *Paragonimus westermani* were examined. The spleen cell culture supernatants were assayed for TH1-specific IFN-γ and TH2-specific IL-4. Cytokine responses for IL-4 peaked at three days (410 ± 60.9 pg/ml), persisted at a high level until the second week (343 ± 59.0 pg/ml), and then decreased slowly four and six weeks after infection. IFN-γ production by splenocytes only increased during the first week (151 ± 32.3 pg/ml) and declined abruptly after the second week of infection. IFN-γ production by splenocytes of infected mice was not observed during the sixth week of infection. In addition, serum IL-4 and IFN-γ were measured. Serum IL-4 was not detected in substantial quantity until four to six weeks after infection. The time course of serum IL-4 was not correlated with that of IL-4 production by splenocytes. Serum IFN-γ was undetectable during the entire course of infection. These results suggest that TH2 cytokine responses, rather than TH1, predominate in mice infected with *P. westermani*.

Key words: interferon-γ, interleukin-4, *Paragonimus westermani*

INTRODUCTION

Elevated serum IgE is one of the most characteristic immune responses in helminthic infections (Radermerker *et al.*, 1974). Elevated IgE levels were shown to be controlled by IL-4 (Finkelman *et al.*, 1986), which is produced by TH2 cells (Mossmann *et al.*, 1986). Dominant TH2 cytokine responses have been reported in many helminthic infections, e.g., *Nippostrongylus braziliensis* (Coffman *et al.*, 1989), *Schistosoma mansoni* (Grzych *et al.*, 1991) and *Trichuris muris* (Else and Grencis, 1991). TH1 cytokine responses occur early in the infection while TH2 cytokine responses peaked during the late stages of the infection in mice infected with *S. mansoni* (Grzych *et al.*, 1991). Therefore, TH2 cytokine responses in helminthic infections are more likely to vary with the species of infecting helminths as well as the different stages of the infection.

P. westermani is a zoonotic parasite. Ingested metacercarial larvae migrate from the intestine to the lung in the definitive hosts. Mice have been regarded as a nonpermissive host for *P. westermani* parasites (Habe, 1978). Although most of the *P. westermani* parasites were recovered from abdominal muscles of mice during the infection (Min *et al.*, 1993), the level of IgE and number of eosinophil leukocytes in the pheripheral blood were elevated similarly as shown for other helminth infections.

Few studies on TH cytokine responses of splenocytes in *P. westermani* infection have been reported. In this study, TH cytokine

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responses of splenocytes in mice infected with *P. westermani* were observed for comparison with other helminthic infections.

MATERIALS AND METHODS

Parasites and experimental animals

Metacercariae of *P. westermani* were separated from crayfish, *Cambaroides similis*, collected at Wando-gun, Chollanam-do, Korea. Male, 5-7-week old BALB/c mice, were inoculated orally with 20 metacercariae. Age-matched mice were used as non-infected controls.

Assays of IL-4 and IFN-γ

Infected mice were killed by cervical dislocation and their spleens removed aseptically. Mononuclear cells were prepared by lysing erythrocytes with 0.17 M Tris-buffered ammonium chloride solution and washed three times with sterile DMEM (Gibco, NY). The cells, pooled from 4 infected mice and 4 non-infected age-matched controls, were resuspended in tissue culture medium [DMEM containing glucose (4.5 g/L), 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM glutamin, 30 mM HEPES and 5×10^{-5} 2-ME] at 4×10^6/ml and incubated at 37°C and 5% CO_2 alone or in the presence of Con A (10 μg/ml, Sigma, St. Louis) in 96 well microtiter plates (Costar, Cambridge, MA). After 24 hours, the supernatant was collected for IFN-γ and IL-4 measurement and stored at -70°C until used. Cytokine levels were expressed as the amount produced under experimental conditions after substracting the cytokine released by controls cultures containing media alone.

To assay for serum IFN-γ and IL-4 levels, blood was collected from the retro-venous plexus of infected mice. Sera were stored at -20°C until used.

ELISA for IFN-γ and IL-4

Cytokine levels in spleen cell culture supernatants and sera were measured by ELISA (Genzyme, Maine). The amounts of cytokine (pg/ml) were calculated by reference to standard curves. The ELISA kit used was sensitive to 5 pg/ml.

Statistical analysis

The Student's t-test was used to determine similarities or difference between groups.

RESULTS

Cytokine production of cultured splenocytes during the course of infection with *P. westermani*.

IL-4 production of infected mice showed a peak at 3 days after infection (410 ± 60.9 pg/ml) and persisted higher levels at two weeks (343 ± 59.0 pg/ml) after infection (Table 1). IL-4 production decreased at four weeks (200 ± 28.3 pg/ml) and six weeks (99 ± 15.2 pg/ml) after infection. IL-4 production of non-infected controls ranged from 122 ± 37.0 to 154 ± 21.2 pg/ml throughout the study (Table

Table 1. Cytokine levels (pg/ml) in splenocyte culture supernatant of mice infected with *Paragonimus westermani*

	Infected mice[a](n = 4)		Non-infected mice[a](n = 4)	
	Interferon-γ	Interleukin 4	Interferon-γ	Interleukin 4
Day 3	ND[b]	410 (60.9)[d]	ND	ND
Week 1	151 (32.3)[c][d]	260 (26.9)[d]	75 (20.1)	140 (27.0)
Week 2	46 (11.8)	343 (59.0)[d]	68 (13.6)	136 (18.5)
Week 4	83 (15.0)	200 (28.3)	65 (15.1)	154 (21.2)
Week 6	< 5	99 (15.2)	80 (19.3)	122 (37.0)

[a]The spleen cells from each group were pooled and stimulated with Con A. Supernatants were collected from cultures after 24 hr and IFN-γ and IL-4 levels determined.

[b]ND: not done

[c]Each value represents the mean (SE) of dupliate assays.

[d]Cytokine levels of infected mice are much higher than those of non-infected mice

Table 2. Serum cytokine level (pg/ml) in mice infected with *Paragonimus westermani*

	Infected mice (n = 4)		Non-infected mice (n = 4)	
	Interferon-γ	Interleukin 4	Interferon-γ	Interleukin 4
Week 1	< 5	< 5	< 5	< 5
Week 2	< 5	15 (2.0)[a]	< 5	< 5
Week 4	< 5	94 (40.5)[b]	< 5	< 5
Week 6	< 5	313 (54.3)[b]	< 5	< 5

[a]Each value represents the mean (SD)
[b]Student's t-test ($P < 0.05$)

1).

IFN-γ production in infected mice demonstrated higher than normal levels only during the first week of infection (151 ± 32.3 pg/ml). IFN-γ production declined after two weeks and, unlike the controls, was undetectable during the sixth week of infection (< 5 pg/ml)(Table 1). IFN-γ production of non-infected controls ranged from 65 ± 15.1 to 80 ± 19.3 pg/ml throughout the study (Table 1).

Serum cytokine levels in *P. westermani*-infected mice

Serum IL-4 of infected mice was detected at 2 weeks after infection (16 ± 2.0 pg/ml) and increased significantly ($P < 0.05$) at four (94 ± 40.5 pg/ml) and six weeks (313 ± 54.3 pg/ml)(Table 2). Serum IL-4 of non-infected controls was undectable (< 5 pg/ml) throughout the study (Table 2).

Serum IFN-γ of both infected and control mice was undetectable (< 5 pg/ml) throughout the study (Table 2).

DISCUSSION

Mice infected with *P. westermani* have been previously shown to have elevated levels of total IgE (Min *et al.*, 1993). The IgE response in *Schistosoma mansoni* infections has been demonstrated to be controlled by cytokine IL-4 (Sher *et al.*, 1990).

The time course of IL-4 production in splenocytes of *P. westermani*-infected mice in the present study was different from that observed for many other helminthic infections. In infections with *S. mansoni*, IL-4 production began at six weeks after infection when eggs were deposited in tissues. After eight weeks, the production gradually decreased, suggesting that the initial contact with gut epitherial tissue of the eggs was the primary stimulus for the significantly greater production of IL-4. The low production of IL-4 during the later course of infection indicated a chronic infection (Grzych *et al.*, 1991). Difference in the IL-4 responses may be related to the different life cycles and biology of the parasite and host susceptibility. In this study, high IL-4 production during the early onset of infection may be associated with high larval migration activity from the intestine to the muscles via the peritoneal cavity. In addition, declining IL-4 production in later course of infection may be due to greatly reduced migratory activity of larvae through abdominal tissues.

In the present study, IFN-γ production by splenocytes showed a similar pattern with IL-4. In response to Con A, the level of IFN-γ increased only during the first week of infection and then decreased thereafter. However, the production of IFN-γ was much lower than that of IL-4. In this study, the reason for no or low production of IFN-γ of splenocytes is uncertain. Low production of IFN-γ may be due to the inhibitory effect of IL-10 (Silva *et al.*, 1992). Further studies on the regulatory mechanisms of IFN-γ in mice infected with *P. westermani* should be investigated.

The role of IL-4 and IFN-γ cytokines in protective immunity is still unknown. Recent studies on the role of cytokines in host protection against helminthic parasites have been reported (Urban *et al.*, 1991, 1993; Else *et al.*, 1994; Ramaswamy *et al.*, 1994). High IFN-γ production in mice and rats infected

with *T. spiralis* may be related to greater resistance to *T. spiralis* infections (Pond *et al.*, 1989; Ramaswamy *et al.*, 1994). High IL-4 production in mice infected with *Trichuris muris* corresponded to worm expulsion (Else *et al.*, 1994).

Serum IL-4 was detectable at the second week of infection and increased significantly after four weeks of infection. However, circulating IFN-γ was not detected in any serum samples collected during the infection. These results are similar to those obtained in mice infected with *S. mansoni* (Amiri *et al.*, 1994). Also, the time course of serum IL-4 coincides with that of total IgE previously reported for *P. westermani*-infected mice.

In summary, this study suggests that TH2 cytokine responses rather than TH1 predominate in mice infected with *P. westermani*. However, further studies are needed to determine the role of TH cytokines in protective immunity to *P. westermani* infections.

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=초록=

폐흡충 감염 마우스에 있어 비장세포에서 분비되는 interferon-γ 및 interleukin-4의 생산

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폐흡충(*Paragonimus westermani*) 감염시 일어나는 TH cytokine 반응을 알아보고자 마우스에 폐흡충 피낭유충을 감염시킨 후 비장세포를 Con A로 자극하여 TH1-specific cytokine인 IFN-γ와 TH2-specific cytokine인 IL-4의 생산량을 감염시기별로 효소표식 면역검사법으로 측정하였다. 폐흡충 감염 마우스의 비장세포에서 생산되는 IL-4는 감염 후 3일(410 ± 60.9 pg/ml)에 최고치에 도달한 후 2주(343 ± 59.0 pg/ml)까지 대조군에 비해 높게 유지되었으나 감염 후 4주에는 감소되기 시작하여 6주에는 대조군의 생산량과 비슷하였다. 한편 폐흡충 감염 마우스의 비장세포에서 생산되는 IFN-γ는 감염 후 1주(151 ± 32.2 pg/ml)에만 대조군에 비해 높게 증가되었을 뿐 2주부터 감소되기 시작하여 감염 후 6주에는 전혀 측정되지 않아 오히려 대조군의 생산량보다도 적었다. 또한 폐흡충 감염 마우스의 혈청 내 IL-4의 양은 감염 후 4주부터 6주에 대조군에 비해 높게 증가되었으나 혈청내 IFN-γ의 양은 전 실험기간을 통해 측정되지 않았다. 이상의 결과를 종합할 때 폐흡충 감염마우스에서는 IFN-γ보다는 IL-4가 증가되는 TH2 cytokine 반응이 주로 일어남을 알 수 있었다.

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