

Changes of IgE production, splenic helper and suppressor T lymphocytes in mice infected with *Paragonimus westermani*

Duk-Young Min*, Jae-Sook Ryu and Myeong-Heon Shin

Department of Parasitology, College of Medicine, Hanyang University, Seoul 133-791, Korea

Abstract: Effects of *Paragonimus westermani* infection were observed in mice on the change of serum IgE level, the number of peripheral eosinophils and the distribution of Thy 1.2⁺ (CD3), L3T4⁺ (CD4), and Lyt-2⁺ (CD8) splenic T lymphocytes without mitogen stimulation. BALB/c mice were infected with 20 metacercariae of *P. westermani* each. Total serum IgE increased at 3 weeks after the infection and reached a peak on week 4 and maintained high levels of IgE until the 23rd week. Peripheral eosinophil numbers increased at the second week and attained peak level on week 9. The frequency of L3T4⁺ (CD4) and Lyt-2⁺ (CD8) T lymphocytes decreased slightly until 4 weeks after the infection, but not significantly. Absolute number of L3T4⁺ and Lyt-2⁺ T lymphocytes, and the ratio of L3T4/Lyt-2 were not markedly changed over the period of observation. The frequency of Thy 1.2⁺ (CD3) T lymphocytes in the infected group slightly decreased until 4 weeks after the infection and showed significant reductions at the 2nd and 4th week of the infection ($p < 0.05$).

Key words: *P. westermani*, IgE antibody, eosinophil, splenic lymphocytes

INTRODUCTION

Elevation of IgE production and peripheral eosinophilia are major immunologic hallmarks of helminthic infection (Radermerker *et al.*, 1974; Jarrett *et al.*, 1976; Rousseaux-Prevost *et al.*, 1977). These responses are stimulated by cytokines (IL-4 and IL-5) of Th2 cells, and administration of either anti-IL-4 or anti-IL-5 antibodies decreased IgE level or the number of eosinophils in the peripheral blood in mice infected with *Nippostrongylus brasiliense* (Coffman *et al.*, 1989).

In experimental murine paragonimiasis, IgE antibody was highly elevated (Shin *et al.*, 1991) and this immunoglobulin had a major role in

antibody dependent cell-mediated cytotoxicity (ADCC) on metacercariae of *P. westermani* *in vitro* (Min *et al.*, 1990).

In murine giardiasis cytotoxic/suppressor T lymphocytes in the epithelia and the lamina propria of intestine were more abundant than helper T cells at the early or acute stage. Also, in patients of trichinosis, the number of CD4⁺ T cells in the peripheral blood was increased relatively to the number of CD8⁺ cells and a high ratio of CD4⁺/CD8⁺ ratio was recorded (Sato & Shiroma, 1989). However, it is still unknown the distribution of splenic lymphocyte subset in mice infected with *P. westermani* by time course of the infection. The increased splenic lymphoproliferation of mice infected with *P. westermani* was documented after the stimulation with same parasite antigen (Min *et al.*, 1992).

This study was carried out to observe the serum IgE level, the number of eosinophil

* Received July 7 1993, revised Aug. 2 1993.

• This paper was supported by NON DIRECTED RESEARCH FUND, Korea Research Fund, 1991.

* Corresponding author

leukocytes in the peripheral blood and the distribution of splenic lymphocyte subsets in *P. westermani*-infected mice in aspects of cytotoxic/suppressor and helper T lymphocytes.

MATERIALS AND METHODS

1. Parasites: Metacercariae of *P. westermani* were separated from crayfish, *Cambaroides similis*, collected at Wando-gun, Chollanam-do, and used for infection to mice.

2. Experimental animal: Five- to 6-week-old BALB/c mice were used. Each mouse was inoculated orally with 20 metacercariae. Age-matched mice were used as the non-infected control.

3. Serum: Blood was collected from retro-venous plexus of mice. Serum was stored at -20°C until used.

4. IgG antibody measurement by ELISA: ELISA was carried out using adult worm antigen (AWA) and excretory-secretory antigen (ESA) as antigen. The methods employed were same as those described previously (Shin *et al.*, 1991).

5. Total IgE determination by ELISA: The methods applied were same as those described previously (Shin *et al.*, 1991). In brief, anti-mouse IgE monoclonal antibody (Pharmagen, CA) was coated onto 96 well flat-bottom microtitre plates at an optimum concentration. The diluted normal or infected sera were then added to wells and incubated at 37°C for 1 hr. Biotin-conjugated anti-mouse IgE polyclonal antibody (Bio design, ME) was added to each well and incubated at 37°C for 1 hr. HRP-conjugated antibiotin antibody (Vector Lab., CA) was then added. To determine IgE concentration, standard mouse IgE (kappa chain, Pharmigen, CA) was used at various concentrations for obtaining the standard curve ($Y = 0.15 X + 0.092$, $r=0.90$).

6. Quantitation of eosinophil leukocytes: Blood was collected from retro-venous plexus or heart of mice. Blood was mixed with

Phloxine diluted solution (propylene glycol 50ml, 1% phloxine 10ml, 10% sodium carbonate 1 ml, D.W. 40ml) and the mixture was placed into WBC pippete. Eosinophil counts were determined with haemocytometer.

7. Analysis of splenic T lymphocyte subsets by flow cytometry

1) Preparation of mononuclear cells: Spleen was chopped in PBS containing 0.1% sodium azide. Cell suspensions were layered over Ficoll-Paque (Pharmacia, NJ) and centrifuged at $400 \times g$ for 30 min. Mononuclear cells were collected and washed 3 times in PBS. Spleen cells were used at a concentration of 5×10^6 /ml.

2) Detection of splenic cell subpopulations: For detection of T cell subsets, the following monoclonal antibodies were used; PE-conjugated anti-mouse L3T4 monoclonal antibody for T-helper, FITC-conjugated anti-mouse Lyt-2 monoclonal antibody for T-cytotoxic/suppressor and FITC-conjugated anti-mouse Thy 1.2 monoclonal antibody for Pan T. For detection of B cell, anti-mouse I-A^d monoclonal antibody and FITC-conjugated anti-mouse IgG_{2a} were used. All reagents were purchased from Becton Dickinson (CA). To 25 μ l cell suspension (1×10^6 /ml), each of above mentioned monoclonal antibodies were added to detect individual cellular subpopulation.

The cells were incubated for 30 min at 4°C and washed 2 times in PBS and then fixed with 1% paraformaldehyde. The number of cell populations were determined by flow cytometric analysis using EPICS C fluorocytometer (Coulter, FL).

8. Distribution of metacercariae of *P. westermani* in mice:

Two mice infected with *P. westermani* were sacrificed (on week 1,2,3,5, 7,9 and 13). Each organ (muscle, lung, heart, liver, spleen, kidney, stomach and intestine) was isolated and chopped in normal saline and then incubated for 1 to 2 hr at room temperature. Recovered worm were washed in normal saline and then fixed, stained and counted.

9. Statistical analysis: The Student's t-test was used to determine the significance of

result difference between the groups.

RESULTS

1. *P. westermani*-specific IgG antibody level:

The specific IgG level slightly increased at 4 weeks after the infection and reached a peak on week 9. The high level was maintained until 23 weeks of observation period. No differences were observed in the antibody levels using AWA or E-S antigen (Fig. 1).

2. Determination of total serum IgE:

Total IgE level increased at 3 weeks after the infection (O.D.; 0.35). The O.D. value was 0.72, 0.68, 0.72, 0.67 and 0.65 on week 4, 5, 7, 9 and 23 respectively (Fig. 2).

Serum IgE level of the infected group was

elevated on week 3 (2.1 $\mu\text{g/ml}$) and reached a peak on week 4 (6.2 $\mu\text{g/ml}$) and the high value was maintained until 23 weeks of infection. Throughout the experimental period non-infected control group showed total IgE levels below 1 $\mu\text{g/ml}$.

3. Quantitation of the number of eosinophils in the peripheral blood:

The number of peripheral eosinophil leukocytes of the infected group increased at 2 weeks after the infection (366.7/mm³) and showed a peak at 9 weeks after the infection (664.1/mm³), then began to be lowered on week 13 (414.8/mm³) and on week 23 (116.6/mm³). Mean number of peripheral eosinophils was 66.6/mm³ (range; 51.8/mm³-86.9/mm³) in non-infected group (Fig. 3).

4. Splenic lymphocyte subsets:

The frequency of L3T4⁺ and Lyt-2⁺ T lymphocytes in the infected group decreased slightly until 4 weeks after the infection. However the difference was not significant throughout the experiment between the two groups (Table 1, P>0.05). The L3T4⁺/Lyt-2⁺ ratio in the infected group did not show remarkable change throughout the experiment. The frequency of Thy 1.2⁺ T lymphocytes in the infected group decreased until 4 weeks after the infection, and showed notable reductions especially on week 2 and 4 of the infection (Table 1, P<0.05). The frequency of B lymphocytes of the infected group was not strikingly different from that of control (Table 1, P>0.05).

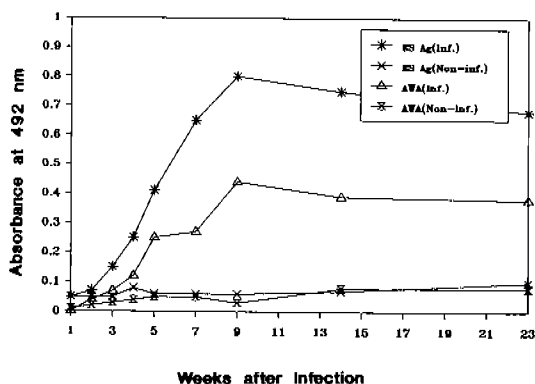


Fig. 1. Serum IgG antibody levels in mice infected with *P. westermani*.

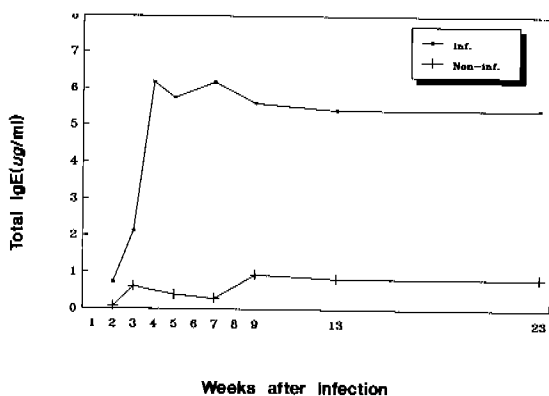


Fig. 2. Total IgE levels in sera of mice infected with *P. westermani*.

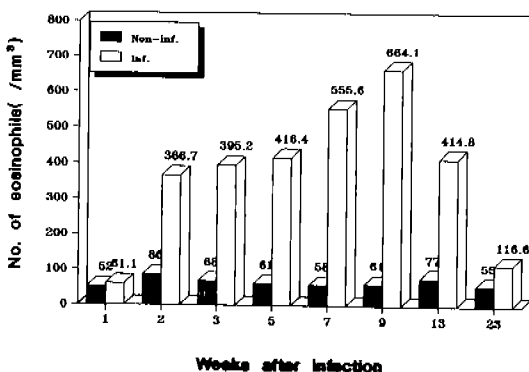


Fig. 3. Number of peripheral blood eosinophils in mice infected with *P. westermani*.

Table 1. Frequency (%) of positive cells in the spleen of mice infected with *Paragonimus westermani* by fluorocytometry

Lymphocyte subset	Days after <i>Paragonimus westermani</i> infection									
	3d	1w	2w	3w	4w	7w	9w	12w	23w	
Th										
Exp.	15.8 ± 6.76 ^{a)}	16.6 ± 1.49	20.8 ± 1.70	18.1 ± 2.82	18.5 ± 1.67	17.1 ± 2.31	20.6 ± 1.80	28.1 ± 0.04	20.6 ± 2.31	
Cont.	15.3 ± 5.83	19.6 ± 3.96	20.8 ± 2.19	20.8 ± 2.20	19.2 ± 2.24	16.7 ± 1.47	19.3 ± 0.93	25.9 ± 4.61	22.9 ± 0.78	
Ts										
Exp.	8.0 ± 1.27	6.0 ± 1.39	8.5 ± 2.08	6.1 ± 0.67	7.5 ± 0.4	11.9 ± 0.41	8.2 ± 1.05	11.3 ± 1.82	9.8 ± 2.76	
Cont.	9.6 ± 5.47	8.3 ± 0.65	8.1 ± 1.95	7.1 ± 1.76	9.0 ± 1.6	10.4 ± 1.48	8.4 ± 1.70	10.3 ± 0.47	8.8 ± 0.51	
Pan T										
Exp.	24.6 ± 1.41	24.4 ± 1.11	28.6 ± 1.35 ^{b)}	23.5 ± 4.79	29.1 ± 0.71 ^{b)}	28.5 ± 4.87	29.0 ± 3.67	35.6 ± 0.07	41.3 ± 4.88	
Cont.	25.6 ± 1.25	28.8 ± 7.34	32.7 ± 2.40	27.4 ± 1.44	36.0 ± 0.87	27.1 ± 4.08	29.8 ± 1.56	36.7 ± 5.20	39.8 ± 1.62	
B										
Exp.	N.D.	54.3 ± 9.66	N.D.	42.7 ± 5.35	N.D.	51.9 ± 5.35 ^{c)}	N.D.	55.6 ± 4.53	N.D.	
Cont.	N.D.	48.8 ± 7.94	N.D.	48.7 ± 5.41	N.D.	53.9 ± 5.67 ^{c)}	N.D.	58.0 ± 3.90	N.D.	
Th/Ts ratio	1.98	2.77	2.45	2.97	2.47	1.44	2.51	2.49	2.10	
Cont.	1.59	2.36	2.57	2.93	2.13	1.61	2.30	2.51	2.60	

^{a)}Mean ± SD, ^{b)}p < 0.05, ^{c)}6th week of infection, d: day, w: week. Th: L3T4 helper T lymphocytes. Cont.: control group. Ts: Lyt-2 cytotoxic/suppressor T lymphocytes. Exp.: experimental group. Pan T: Thy 1.2T lymphocytes. B: I-Ad B lymphocytes.

5. Analysis of spleen weight: Weight of spleen in the infected group was considerably increased during the course of the infection, especially on week 2, 3, 5 and 7 comparing with those of non-infected control (Fig. 4, $P < 0.05$).

6. Distribution of *P. westermani* in mice: One hundred and seventy four worms were recovered from 15 infected mice and the recovery rate was 58%. Among 174 worms recovered, 161 worms were found in muscle, 13 in liver, abdominal cavity and pleural cavity. No worm was found in lung. At one week after the infection 80% of recovered worms were found in muscles under diaphragm

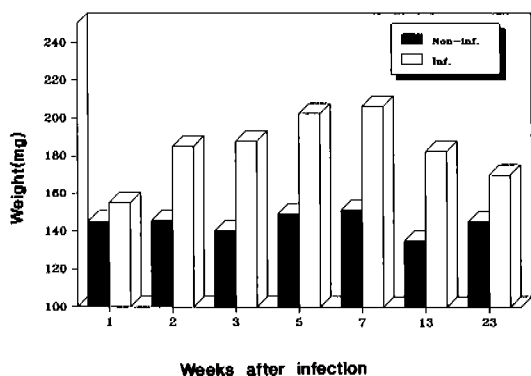


Fig. 4. Spleen weight of mice infected with *P. westermani*.

(Table 2). Motility of the most of recovered worms at early time of the infection was active. But a few of worms had a good motility at the later stage of the infection. All of recovered worms were immature and almost same in size regardless of recovered sites. The average length of worm ranged $1036 \pm 231.4 \times 443.4 \pm 54.3$ on 13th week after the infection.

DISCUSSION

Total serum IgE level of mouse infected with metacercariae of *P. westermani* increased at the 3rd week of the infection and reached a peak at 4 weeks and maintained higher level until the 23rd week. In our experiment, the worm recovery was not variable until 13 weeks after the infection. Min *et al.* (1980) and Pfister *et al.* (1983) reported that total serum IgE level increased at earlier infection and was sustained until the later infection in rats infected with *C. sinensis* and *F. hepatica*. Ikeda and Fujita (1980) showed that IgE antibody production in rats infected with *P. ohirai* was dependent upon routes of the infection. Oral and intraperitoneal infection with metacercariae induced IgE elevation during larval migration to abdominal cavity and liver, however intrapleural infection failed IgE production due to lack of migratory phase. In helminthiasis, in general, high serum IgE levels were maintained by the active infection of worms and these levels were remarkably reduced within months after the elimination of

Table 2. Recovery of immature worms in mice infected with *P. westermani*^{a)}

Weeks after infection	No. of mice examined	No. of worms recovered from					Total (%)
		Muscle		Abdominal cavity	Liver	Pleural cavity	
		Upper D. ^{b)}	Lower D. ^{c)}				
1	3	3	33	1	4	1	42(70.0)
2	2	12	13				25(62.5)
3	2	11	12				23(57.5)
5	2	14	8		1		23(57.5)
7	2	5	13	1		2	21(52.5)
9	2	5	6	2			13(32.5)
13	2	14	12		1		27(67.5)
Total	15	64	97	4	6	3	174(58.0)

^{a)} Each mouse was inoculated orally with 20 metacercariae of *P. westermani*. ^{b)} Upper D.: Upper parts of diaphragm. ^{c)} Lower D.: Lower parts of diaphragm.

worm (Jarrett *et al.*, 1976; Pfister *et al.*, 1983).

Eosinophilia is one of the characteristic immune responses in helminthiasis (Ansari & Williams, 1976; Ackerman *et al.*, 1981; Sugane & Oshima, 1982). In this study the number of eosinophils increased at 2 weeks of the infection and showed a peak on week 9 and slowly declined thereafter. On week 23 eosinophils were remarkably reduced, but still significantly higher than uninfected controls. Sugane (1988) observed that the number of peripheral eosinophils of mice infected with *Ascaris suum* revealed a peak on week 2 and then declined within on week 8 with subsequent disappearance of larval migration. The results from this study give a suggestion that eosinophilia in helminthiasis has a close relationship with migratory activity of the worms.

There were many reports on changing patterns of lymphocyte proliferation in parasitic infections. In murine schistosomiasis mansoni, splenic L3T4⁺ subset was the major soluble egg antigen (SEA)-specific proliferative population (Yamashita & Boros, 1990). In acute toxoplasmosis, helper T cell was parasite-specific proliferative population (Sharma, 1990). Therefore, CD4⁺ lymphocytes were thought to be the principal effector T cells mediating both protective and immunopathological responses against helminth and protozoan infections (Sher & Coffman, 1992).

In murine paragonimiasis the increased proliferation of lymphocytes by antigen stimulation *in vitro* was observed (Min *et al.*, 1992). This result suggested that *P. westermani* infection could induce lymphocyte activation. In this study the distribution of splenic T lymphocyte subsets in mice infected with *P. westermani* was examined in the absence of mitogenic or antigenic stimulation. The numbers of Thy 1.2⁺ (CD3) T cells were significantly lower in infected mice than controls at 2 and 4 weeks after the infection, although the absolute number of L3T4⁺ (CD4) and Lyt-2⁺ (CD8) T cell and the CD4/CD8 ratio in the infected group did not show the significant change over the period of observation. These findings assumed that the lack of challenge stimulation with mitogen could not induce the differentiation of

splenocyte *in vitro*. Sztein *et al.* (1990) also reported that *T. cruzi* could not alter the number of CD3⁺, CD4⁺ and CD8⁺ cells in resting cells.

This study was aimed to investigate the relationship between IgE elevation and the percentage distribution of T_h cells expressing CD4 surface marker. Unexpectedly the results of this study showed that percentage of Thy cells expressing CD4 surface marker of the infected mice was not related with the time course of IgE elevation. Seven weeks after the infection the expression of CD4⁺ cells increased slightly but there was no differences between two groups. These findings suggested that cytokine production of the activated cells was able to provide more substantial help for IgE production than T cell itself (Maggi *et al.*, 1988). It should therefore be examined the cell subpopulation of spleen and cytokine production after the stimulation with worm antigen or mitogen.

In conclusion, *P. westermani* infection in mice resulted in prominent increase of serum IgE value and the number of peripheral eosinophil leukocytes, however the infection did not affect the changes of Thy 1.2⁺, L3T4⁺ and Lyt-2⁺ splenic T lymphocytes subpopulation in resting stage.

ACKNOWLEDGEMENT

The authors would like to thank Mr. H.K. Choi for technical assistance.

REFERENCES

- Ackerman SJ, Gleich GJ, Weller PF, Ottensen EA (1992) Eosinophilia and elevated serum levels of eosinophil major basic protein and Charcot-Leyden crystal protein (lysophospholipase) after treatment of patients with Bancroft's filariasis. *J Immunol* **127**: 1093-1098.
- Ansari A, Williams JF (1976) The eosinophilic response of the rat to infection with *Taenia taeniaeformis*. *J Parasitol* **62**: 728-736.
- Coffman RL, Carty J (1986) A T cell activity that enhances polyclonal IgE production and its inhibition by interferon- γ . *J Immunol* **136**: 949-954.
- Coffman RL, Seymour BWP, Hudak S, Jackson J,

- Rennick D (1989) Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* **245**: 308-310.
- Ikeda T, Fujita K (1980) IgE in *Paragonimus ohirai*-infected rats: Relationship between titer, migration route and parasitic age. *J Parasitol* **66**(2): 197-204.
- Jarrett EEE, Haig DM, Bazzin H (1976) Time course studies on rat IgE production in *N. brasiliensis* infection. *Clin Exp Immunol* **24**: 346-351.
- Maggi E, Del Prete GF, Tiri A, Macchia D, Parronchi P, Ricci M (1988) T cell clones providing helper function for IgE synthesis release soluble factor (s) that induce IgE production in human B cells: possible role for interleukin 4 (IL-4). *Clin Exp Immunol* **73**: 57-62.
- Min DY, Ahn MH, Kim KM, Leem MH, Park SY (1990) The effects of antibodies and complement in macrophage-mediated cytotoxicity on metacercariae of the lung fluke, *Paragonimus westermani*. *Korean J Parasit* **28**: 91-100 (in Korean).
- Min DY, Shin MH, Choi R (1992) Blastogenesis of *Paragonimus westermani* infected mouse spleen cell. *Korean J Parasit* **30**: 43-48 (in Korean).
- Min DY, Soh CT, Capron A (1980) Serum IgE Level in *Clonorchis sinensis* Infected Rat and Isolation of the Allergen. *Yonsei J Med Sc* **13**: 94-106 (in Korean).
- Pfister K, Turner K, Currie A, Hall E, Jarrett EEE (1983) IgE production in rat fascioliasis. *Parasite Immunol* **5**: 587-593.
- Radermecker M, Bekhti A, Doncelet E, Salmon J (1974) Serum IgE levels in protozoal and helminthic infections. *Int Archs Allergy Appl Immunol* **47**: 285-295.
- Rousseaux-Prevost R, Capron M, Bazin H, Capron A (1977) IgE in experimental schistosomiasis. *Immunology* **33**: 501-505.
- Sato Y, Shiroma Y (1989) Peripheral lymphocyte subsets and their responsiveness in human strongyloidiasis. *Clin Immunol Immunopathol* **53**: 430-438.
- Sharma SD (1990) Immunology of Toxoplasmosis in Modern Parasite Biology. Cellular, Immunological and Molecular Aspects. pp 184-199.
- Sher A, Coffman RL (1992) Regulation of immunity to parasites by T cells and T cell-derived cytokines. *Annu Rev Immunol* **10**: 385-409.
- Sher A, Coffman RL, Hieng S, Scott P, Cheever AW (1990) Interleukin 5 is required for the blood and tissue eosinophilia but not granuloma formation induced by infection with *Schistosoma mansoni*. *Proc Natl Acad Sci USA* **87**: 61-65.
- Shin MH, Ryu JS, Min DY (1991) Serum IgE levels in rats infected with *Paragonimus westermani*. *Korean J Parasit* **29**: 397-401 (in Korean).
- Sugane K (1988) Eosinophilia in *Ascaris suum*-reinfected mice. *J Helminthol* **62**: 51-57.
- Sugane K, Oshima T (1982) Recovery of large numbers of eosinophil from mice infected with *Toxocara canis*. *Am J Trop Med Hyg* **29**: 799-802.
- Sztein MB, Cuna WR, Kierszenbaum F (1990) *Trypanosoma cruzi* inhibits the expression of CD3, CD4, CD8 and IL-2R by mitogen-activated helper and cytotoxic human lymphocyte. *J Immunol* **144**: 3558-3662.
- Vinayak VK, Khanna R, Kum K, Nain CK (1989) Functional characterization of intestinal intraepithelium and lamina propria lymphocytes from *Giardia lamblia* infected mice. *Indian J Med Res* **89**: 271-281.
- Yamashita T, Boros DL (1990) Changing patterns of lymphocyte proliferation, IL-2 production and utilization, and IL-2 receptor expression in mice infected with *Schistosoma mansoni*. *J Immunol* **145**: 724-731.

=국문초록=

폐흡충(*Paragonimus westermani*) 감염이 흰쥐의 IgE 생성 및 비장림프구 아군분포에 미치는 영향

한양대학교 의과대학 기생충학교실

민득영, 류재숙, 신명현

폐흡충 피낭유충을 BALB/c 마우스에 경구 감염시킨 후 마우스를 시기별(3일, 1주, 2주, 3주, 4주, 5주, 7주, 9주, 13주 및 23주)로 희생시켜 말초혈액내 호산구수와 혈청내 IgE 항체가를 측정하는 한편 비장 림프구를 분리하여 림프구 아군(helper T, cytotoxic/suppressor T, pan T)에 대한 단세포군 항체를 결합시켜 EPICS C fluorocytometer로 분석하였다. 폐흡충에 대한 혈청내 총 IgE 항체가는 감염 3주부터 증가되어 감염 4주에 높게 증가되었으며 13주 이후 23주까지 높은 항체가가 유지되었다. 말초혈액내 호산구수는 감염 2주 부터 증가되어 감염 9주에 $664.1/mm^3$ 으로 가장 증가 되었으며 그 이후에는 감소하여 23주에는 정상보다 약간 증가되었다. 폐흡충을 감염시키고 감염 3일, 1주, 2주, 3주, 4주, 7주 및 23주에 T 림프구 아군을 분석한 결과 L3T4 helper T 림프구와 Lyt-2 cytotoxic/suppressor T 림프구의 비율은 감염군에서 감염 초기에 대조군에 비해 약간 감소된 경향을 보였으나 대조군과 유의한 차이를 보이지 않았다. Thy 1.2 pan T 림프구의 비율은 감염 2주와 4주에 대조군에 비해 유의하게 감소되었고(P<0.05) 7주 이후에는 대조군과 비슷하였다. 이러한 결과들로 보아 마우스의 폐흡충감염에서 특이 항원이나 마이토젠의 자극이 없는 비장림프구의 아군분포에 큰 변화가 없는 것으로 생각된다.

(기생충학잡지 31(3): 231-238, 1993년 9월)