

Elevation of Specific IgE Antibody in *Clonorchis sinensis* infection

Duk-Young Min and Chin-Thack Soh

Department of Parasitology, Yonsei University Medical College

Institute of Tropical Medicine, Yonsei University

INTRODUCTION

It has already been recognized that one of the major characteristics of the immune response to helminths infection is to produce immunoglobulin E (=IgE). High human serum IgE levels were demonstrated not only in atopic diseases (Johansson, 1967; Berg and Johansson, 1969) but also in various helminths infections such as ascariasis (Johansson *et al.*, 1968), visceral larva migrans (Hogarth-Scott *et al.*, 1969) and schistosomiasis (Ito *et al.*, 1972; Kojima *et al.*, 1972; Dessaint *et al.*, 1975).

The protective role of IgE in parasitic infection is still questionable. Murray *et al.* (1971) demonstrated that IgE antibodies may play a secondary part by inducing immediate hypersensitivity reaction which help the attack of worms by IgG antibodies in rat infected with *Nippostrongylus brasiliensis*. It has been interpreted that local anaphylaxis helps the "leakage of IgG" into the intestinal lumen and then worms are eliminated, although there have been some disputes (Jones and Ogilvie, 1970). However Capron and his colleagues (1975, 1977, 1978 a,b) demonstrated protective activity of serum IgE and specific IgE to parasites which were shown to have a ability to activate macrophages of normal rats. This activation may enhance the

cytotoxic effects of macrophage to *Schistosoma mansoni* schistosomule.

In the present study, serum IgE level and parasite specific IgE level in human clonorchiasis were determined, and the relationships between each levels and amount of egg deposition in feces of patients have elucidated.

MATERIALS AND METHODS

Collection of sera:

Sera from 21 clonorchiasis patients and 15 healthy individuals were collected at room temperature then kept frozen at -20°C until use.

Egg deposition in feces of clonorchiasis patients were estimated as eggs per gram (EPG) of feces (Table 1).

Quantitative measurement of serum IgE:

The radioimmunosorbent test (RIST) was performed with the commercial kit of Pharmacia Diagnostics (Uppsala, Sweden). Preparation 69/204 for Human Serum IgE, from the WHO International Reference Center for Immunoglobulins, Lausanne, Switzerland was used for standardization. The percentage of radioactivity bound to Sephadex-coupled antibody was read from the standard curve as the concentration of IgE in international unit (IU)/ml.

Quantitation of specific IgE antibody:

Parasite specific serum IgE antibody level was determined with radioallergosorbent technique (RAST).

A soluble antigenic preparation of *Clonorchis sinensis* (Min *et al.*, 1980) was coupled on CNBr activated cellulose. Fifty μl of test serum was

* This study was performed with Research Grant of Ministry of Education, 1982 and with Faculty Research Grant of Yonsei University Medical College, 1980.

mixed in 500 μ l of antigen coupled cellulose suspension and then agitated rotatively overnight at room temperature. After three times of washing with 0.05M phosphate buffered saline(PBS) pH 7.4 containing 0.01% NaN₃, bovine serum albumin(BSA) and Tween 20, 50 μ l of radioactive iodine (I¹²⁵) labelled anti-IgE(8mg/50 μ l) (it was supplied by Prof. Capron, Institut Pasteur, Lille, France) (anti-IgE I¹²⁵) was added and then agitated rotatively overnight at room temperature. After washing 2 times again with PBS pH 7.4 radioactivity of culot was counted. The results were expressed as per cent according to following equation;

$$\frac{\text{radioactivity of test serum} - \text{radioactivity of tube 0}}{2 \times \text{radioactivity of tube 0}} \times 100$$

(Tube 0 means the tube did not contain any human serum)

The means of each group were statistically analysed and compared the significance and correlation.

Table 1. Sex, age and EPG(eggs per gram of feces) of 21 clonorchiasis patients

Case No.	Name	Sex	Age	EPG
1	Moon YT	M	16	8,700
2	Lee KH	M	35	2,400
3	Park YS	M	17	11,200
4	Lim HH	M	12	1,200
5	Cheon H	M	14	5,900
6	Huh JO	M	13	11,500
7	Cho SO	M	14	3,000
8	Kwon SM	M	11	11,700
9	Han JS	F	12	34,900
10	Han KS	M	49	1,100
11	Park SI	F	13	2,200
12	Moon TS	M	16	56,100
13	Beon SJ	F	17	5,000
14	Koh SH	M	41	2,300
15	Han DS	M	40	1,400
16	Han IS	M	61	1,400
17	Kim BS	M	14	69,400
18	Lee YJ	F	17	4,800
19	Kim OS	F	40	17,100
20	Min DY	M	40	200
21	Song OS	F	17	12,100

RESULTS

Serum IgE levels: Serum IgE level in individuals infected with *Clonorchis sinensis* was compared with the serum IgE level in healthy individuals.

The serum IgE levels in 15 healthy individuals were in the range from 51 to 1,966 IU/ml (mean \pm SD: 364 \pm 557 IU/ml).

Among them two cases showed over 700 IU/ml (Table 2). Otherwise serum IgE levels in 21 clonorchiasis were from 388 to 7,546 IU/ml (mean \pm SD: 2,372 \pm 2,293 IU/ml) (Table 3). Five cases among the 21 clonorchiasis revealed less than 700 IU/ml of serum IgE, and showed highly significant elevation (P<0.001) to compare the control group (Table 4).

The levels of specific IgE to *Clonorchis sinensis* in 15 healthy individuals were from 0 to 38.5%, and the mean value was 4.4%.

In two cases who revealed the high serum IgE level also showed high specific IgE levels (Table 2). But in individuals infected with

Table 2. Serum IgE and *Clonorchis* specific IgE levels in 15 healthy individuals

Case No.	Serum IgE(IU/ml)	Specific IgE*(%)
1	91	0
2	93	0
3	621	0
4	56	0
5	179	2.7
6	53	0
7	120	0
8	1,996	38.5
9	375	0
10	152	1.1
11	60	0
12	204	0
13	119	4.3
14	1,292	20.2
15	51	0
Mean \pm SD: 364 \pm 557		Mean: 4.4%

* %:
$$\frac{\text{radioactivity of test serum} - \text{radioactivity of tube 0}}{2 \times \text{radioactivity of tube 0}} \times 100$$

Table 3. Serum IgE and *Clonorchis*-specific IgE levels in 21 individuals infected with *Clonorchis sinensis*

Case No.	Serum IgE(IU/ml)	Specific IgE*(%)
1	2,155	54.0
2	930	33.5
3	5,498	184.2
4	517	6.2
5	2,369	50.4
6	611	0
7	388	0.6
8	3,250	22.1
9	316	7.4
10	1,086	0.3
11	1,365	34.3
12	5,906	138.6
13	947	12.4
14	449	7.6
15	4,366	67.7
16	7,546	184.6
17	6,814	219.4
18	675	12.7
19	1,441	11.0
20	719	14.0
21	2,462	29.1
Mean±SD: 2,372±2,293		Mean: 52.0%

* %: radioactivity of test serum radioactivity of tube 0 / (2 × radioactivity of tube 0) × 100

Table 4. Comparison of serum IgE and *Clonorchis* specific IgE levels between individuals infected with *Clonorchis sinensis* and healthy individuals

	No. tested	Serum IgE* (Mean±SD)	Specific IgE**
Clonorchiasis	21	2,372±2,293 IU/ml	52.0%
Healthy	15	362±557 IU/ml	4.4%

* t=3.28, p<0.001

** t=2.59, p<0.01

Coefficient of correlation between:
 serum IgE and specific IgE, r=0.9451
 serum IgE and EPG, r=0.6059
 specific IgE and EPG, r=0.5693

Clonorchis sinensis the specific IgE levels were from 0 to 219.4%, and the mean value was 52.0%.

In three cases who had low value (<700 IU/ml) of serum IgE, the level of specific IgE

(<5%) was also correlated (Table 3), and were recognized as those of healthy individuals.

However, the difference between infected and healthy groups is statistically significant (P<0.01) (Table 4).

There was close correlation between the serum IgE level and the level of specific IgE to *Clonorchis sinensis* in individuals infected with *Clonorchis sinensis* (coefficient of correlation r=0.9451) and a little correlation between serum IgE and EPG (r=0.6056), and between specific IgE and EPG (r=0.5693) are observed (Table 4).

DISCUSSION

Reaginic antibodies are produced in human infected with helminths (Jarrett, 1972).

The results presented in this study indicate that *Clonorchis sinensis* infection causes an increase of serum IgE and specific IgE in man. The mean values of serum IgE and specific IgE of individuals infected with *Clonorchis sinensis* are about 7 and 13 times of those of healthy group. Before, Johansson *et al.* (1968) reported that the mean value of serum IgE levels in infected children with *Ascaris lumbricoides* was 4,400 ng/ml which was about 5 times of that of the non-infected children. Kojma *et al.* (1972) reported also the increased serum IgE levels in schistosomiasis japonica, paragonimiasis, fascioliasis hepatica and hookworm infection. Meanwhile Dessaint *et al.* (1975) demonstrated high serum IgE and high specific IgE levels in patients with schistosomiasis, and the concentration of IgE antibodies to schistosome were from 2.6 to 5.1% of serum IgE concentration.

The results reported here may provide an possible evidence that raised IgE antibody to *Clonorchis sinensis* are due to host immune response to parasitic antigen. Ishizaka *et al.* (1967) demonstrated a complete parallelism between the elevated IgE antibody and the high Prausnitz-Küstner (P-K) titer of ragweed sensitive patients' sera. Min *et al.* (1980) also reported the high production of serum IgE in rat infected with *Clonorchis sinensis*, and demonstrated the

passively transferable antibodies in rat system by passive cutaneous anaphylaxis(PCA). These homocytotropic antibodies persisted at least 6 weeks in rat. Furthermore the observation of the close correlation between serum IgE level and parasite specific IgE level (coefficient of correlation $r=0.9451$) in this study may provide another possible evidence that parasite is a necessary requirement for IgE antibody production.

In any event, it is clear that the parasites infection shall be the primary factor in the productions of both serum IgE and parasite specific IgE antibodies.

SUMMARY

Serum IgE level and *Clonorchis* specific IgE in individuals infected with *Clonorchis sinensis* were determined by radioimmunosorbent(RIST) and radioallergosorbent technique(RAST) respectively. Highly significant elevations of serum IgE ($P<0.001$) and specific IgE antibodies ($P<0.01$) were observed in sera from individuals with clonorchiasis.

The mean values of serum IgE in individuals with clonorchiasis and healthy individuals were 2,372 IU/ml and 364 IU/ml respectively and specific IgE antibodies of both groups were 52.0 and 4.4%. A close correlation($r=0.9451$) between serum IgE level and specific IgE antibodies were observed and correlation($r=0.6056$) between serum IgE and EPG and between specific IgE and EPG($r=0.5693$) were also observed.

ACKNOWLEDGEMENT

The authors express their sincere thanks to Professor André Capron, Institut Pasteur de Lille, France for supplying materials.

REFERENCES

Berg, T., Johansson, S.G.O. (1969) IgE concentration in children with atopic diseases. A clinical study. *Int. Arch. Allergy*, **36**: 219-239.
Capron, A., Dessaint, J-P, Capron, M., Bazin, H.(1975)

Specific IgE antibodies in immune adherence of normal macrophages to *Schistosoma mansoni* schistosomules. *Nature*, **253**(5491): 474-475.
Capron, A., Dessaint, J-P.(1977) IgE and cells in protective immunity. *Path. Biol.*, **25**(5): 287-290.
Capron, M., Camus, D., Carlier, Y., Figueiredo Jose, F.M., Capron, A. (1977) Immunological studies in human schistosomiasis II. Antibodies cytotoxic for *Schistosoma mansoni* schistosomules. *Am. J. Trop. Med. Hyg.*, **26**(2): 248-253.
Capron, M., Rousseaux, J., Mazingue, C., Bazin, H., Capron, A. (1978a) Rat mast cell-eosinophil interaction in antibody-dependent eosinophil cytotoxicity to *Schistosoma mansoni* schistosomule. *J. Immunol.*, **121**(6): 2518-2525.
Capron, M., Capron, A., Torpier, G., Bazin, H., Bout, D., Joseph, M. (1978b) Eosinophil-dependent cytotoxicity in rat schistosomiasis. Involvement of IgG_{2a} antibody and role of mast cells. *Eur. J. Immunol.*, **8**(2): 127-133.
Dessaint, J.P., Capron, M., Bout, D., Capron, A. (1975) Quantitative determination of specific IgE antibodies to schistosome antigens and serum IgE levels in patients with schistosomiasis (*S. mansoni* or *S. haematobium*). *Clin. Exp. Immunol.*, **20**: 427-436.
Hogarth-Scott, R.S., Johansson, S.G.O., Bennich, H. (1969) Antibodies to *Toxocara* in the sera of visceral larva migrans patients; the significance of raised levels of IgE. *Clin. Exp. Immunol.*, **5**: 619-625.
Ishizaka, K., Ishizaka, T. and Hornbrook, M.M.(1967) Allergen-binding activity of γE , γG , γA antibodies in sera from atopic patients. *In vitro* measurements of reaginic antibody. *J. Immunol.*, **98**: 490-501.
Ito, K., Sawada, T., Sato, S. (1972) Increased serum IgE level in individuals infected with *S. japonicum*, *W. bancrofti* or hook worm, and the changes of treatment in schistosomiasis. *Japan J. Exp. Med.*, **42**(2): 115-123.
Jarrett, E.E.E. (1972) Helminth infection and the biosynthesis of IgE antibodies. *Trans. Roy. Soc. Trop. Med. Hyg.*, **66**: 546.
Johansson, S.G.O. (1967) Raised levels of a new immunoglobulin class (IgND) in asthma. *Lancet*, **II**, 951-953.
Johansson, S.G.O., Mellbin, T., Vahlquist, G. (1968) Immunoglobulin levels in Ethiopian preschool children with special reference to high concentrations of immunoglobulin E(IgND). *Lancet*, **I**: 1118-1121.

Jones, V.E. and Ogilvie, B.M. (1970) Protective immunity to *Nippostrongylus brasiliensis*: the sequence of events which expels worms from rat intestine. *Immunology*, **20**: 549.

Kojima, S., Yokogawa, M., Tada, T. (1972) Raised levels of serum IgE in human helminthiasis. *Am. J. Trop. Med. Hyg.*, **21**(6): 913-918.

Min, D.Y., Soh, C.T. and Capron, A. (1980) Serum

IgE level in *Clonorchis sinensis* infected rat and isolation of the allergen. *Yonsei J. Med. Sci.*, **13** (2): 94-105.

Murray, M., Jarrett, W.F.H., Jennings, F.W. (1971) Mast cells and macromolecular leak in intestinal immunological reactions. The influence of sex of rats infected with *N. brasiliensis*. *Immunology*, **21**: 17-31.

=국문요약=

간흡충증 환자의 혈청내 IgE 및 간흡충 특이 IgE 항체의 상승

연세대학교 의과대학 기생충학교실 및 열대의학연구소
민득영·소진탁

21명의 간흡충 감염환자 및 15명의 건강인으로부터 혈청을 수집하여 혈청내 IgE와 간흡충에 대한 특이 IgE 항체를 RIST와 RAST법을 이용하여 측정하였다.

간흡충 감염환자 및 건강인의 혈청 IgE와 특이 IgE는 각각 2,372 IU/ml과 364 IU/ml 그리고 52.0%와 4.4%로 간흡충 감염자에서 모두 유의하게 상승되어 있었으며 ($p < 0.001$ 및 $p < 0.01$), 혈청 IgE의 상승과 특이 IgE의 상승은 상호 밀접한 관계가 있었고 ($r = 0.9451$), 혈청 IgE와 EPG ($r = 0.6056$), 특이 IgE와 EPG ($r = 0.5693$) 역시 상관 관계가 있었다.

이상의 결과로 보아 간흡충 감염은 인체 혈청내 IgE 및 특이 IgE항체를 상승시키며, IgE 및 간흡충에 대한 특이 IgE는 숙주-기생충간의 면역반응에 관여할 것으로 생각된다.