[Jpn. J. Parasitol., Vol. 35, No. 4, 295–301, August, 1986]

Detection of Specific IgE Antibodies in Guatemalan Onchocerciasis by Enzyme-Linked Immunosorbent Assay (ELISA)

MASATAKA KORENAGA[1], ISAO TADA[1], YOSHIHISA HASHIGUCHI[2], HIROYUKI TAKAOKA[3], MINORU BABA[3], JULIO C. CASTRO[4] AND GUILLERMO E. ZEA F.[4]

(Received for publication; January 6, 1986)

Key words: *Onchocerca volvulus*, adult antigen, IgE antibody, ELISA, Guatemala

Introduction

The production of IgE antibodies is particularly characteristic of the immune response to parasitic helminths. Recently, evidences that IgE antibodies are involved in antibody-dependent cell-mediated cytotoxicity as a mechanism of defense against parasites have accumulated (Capron *et al.*, 1982). In this context, it is important to examine a relationship between the production of IgE antibodies in onchocerciasis patients and various regulatory factors for it. An elevation of the total serum IgE (Somorin *et al.*, 1977; Akiyama *et al.*, 1981), the presence of specific IgE antibodies (Weiss *et al.*, 1982; Kawabata *et al.*, 1983; Ouaissi *et al.*, 1983) and of circulating immune complexes including IgE in sera of patients with onchocerciasis (Steward *et al.*, 1982) have been reported. However, very few investigations have ever been performed on the IgE antibody response relating to the worm burdens, ageing and sex of the patients. We analyzed the relationship between the production of specific IgE antibodies and these factors.

[1]*Department of Parasitic Diseases, Kumamoto University Medical School, Kumamoto 860, Japan.*
[2]*Department of Parasitology, Kochi Medical School, Kochi 781-51, Japan.*
[3]*Division of Medical Zoology, Medical College of Oita, Oita 879-56, Japan.*
[4]*Departamento de Oncocercosis, Servicio Nacional de Erradicacion de la Malaria, Guatemala.*

The study received grants in aid for Special Research Promotion (No. 57123117) and for the Overseas Scientific Research Program (No. 59041050), the Ministry of Education, Science and Culture, Japan.

Materials and Methods

Antigen

Crude extract of the adult worms of *Onchocerca volvulus* recovered from onchocercal nodules of Guatemalan patients was used as an antigen as described previously (Korenaga *et al.*, 1983). Briefly, whole worms were homogenized in 0.05 M carbonate-bicarbonate buffer, pH 9.6, containing 0.02% sodium azide. The homogenate was kept in a refrigerator and was then centrifuged. The supernatant was used as a crude antigen.

Sera

Sera form the following three groups were examined; 301 inhabitants of 4 villages in endemic areas of Chicacao and Chimaltenango Counties in Guatemala, 51 inhabitants of a nonendemic area, Coatepeque, Guatemala in order to evaluate cross-reactions against intestinal helminths, and 11 parasite-free Japanese.

Parasitological examination

The residents were examined by skin snip method and palpation for onchocercal nodules.

Counting microfilariae (mf) by the skin snipping was done according to the method of Tada *et al.* (1985). Fecal samples from inhabitants of both endemic and nonendemic areas were examined to determine the prevalence of intestinal helminths. In the surveyed areas the prevalences of *Ascaris lumbricoides, Trichuris trichiura,* and hookworms were 46–70, 40–72 and 36–45%, respectively. There was no difference in the prevalence of the intestinal helminths infections between onchocerciasis patients and people in the nonendemic area.

Enzyme-linked immunosorbent assay (ELISA)

The procedure for detection of IgE antibody was slightly modified from that described by Abe *et al.* (1985). The flat bottom wells in microtitration plate (Immuno Plate II 96 F, Nunc, Roskilde, Denmark) were sensitized overnight at 4°C with 100 μl of the antigen solution diluted in 0.05 M carbonate-bicarbonate buffer, pH 9.6. Unbound antigen was removed by washing the wells three times the wells with 0.05% (v/v) Tween 20 in saline (saline/T). To each well, 100 μl of 10% normal horse serum (NHS) in saline was added and the plates were incubated at 37°C for 1 hr. Then, 100 μl of serum sample diluted in NHS/saline/T was incubated at 37°C for 3 hr. After washings with saline/T, 50 μl of β-D-galactosidase conjugated sheep antihuman-IgE antibody (Medical and Biological Laboratories, Nagoya, Japan) diluted in NHS/saline/T was added. After an overnight incubation at 25°C, the plates were washed and incubated with 100 μl of the substrate, 0.1% 0-nitrophenyl-β-D-galactoside (Sigma, USA)/1.5 mM $MgCl_2$/0.7% 2-mercaptoethanol/0.05 M phosphate buffer, pH 7.0, for 1.5 hr at 37°C. The reaction was stopped by the addition of 100 μl of 0.2 M Na_2CO_3 and the absorbance values by the optical density (OD) were read by a spectrophotometer at 414 nm.

Optimum concentrations of antigen, serum sample, and conjugate were determined as 10 μg/ml, 1:4 and 1:20, respectively, from the titration experiments.

Statistical analysis

All the statistical analyses in the present work were carried out by Student's t test.

Results

The frequency distribution of IgE values (OD_{414}) of the following three groups is plotted in Fig. 1. The mean value ± SD of inhabitants of endemic areas (Group I-A, n=301)

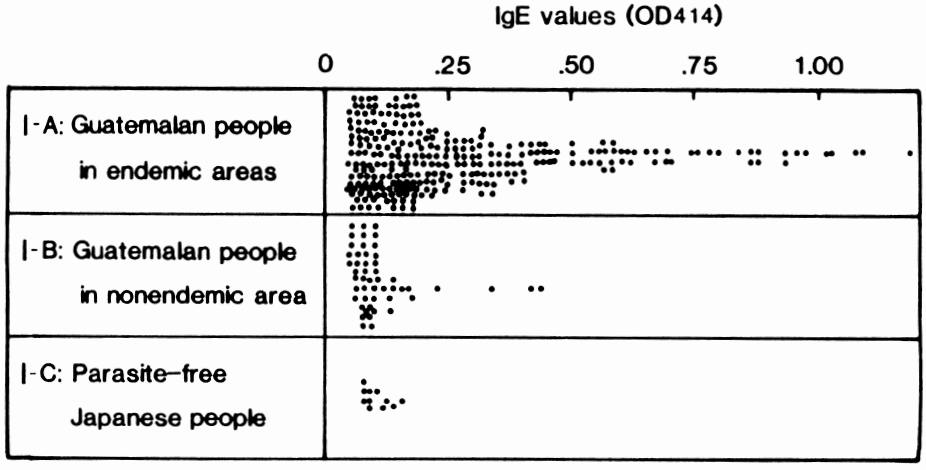

Fig. 1 Distribution pattern of specific IgE values in endemic and non-endemic areas.
I-A vs I-B, I-A vs I-C; significant ($P < 0.005$)
I-B vs I-C; not significant

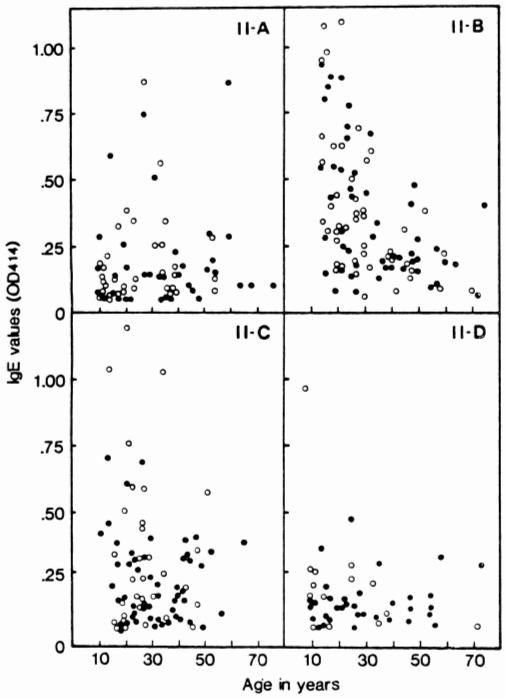

Fig. 2 Distribution pattern of specific IgE values in 4 endemic villages; II-A (El Regalo), II-B (Santa Ines), II-C (Pacayalito) and II-D (Sibaja).
•, male; ○, female

was 0.27 ± 0.22, while those of nonendemic (Group I-B, n=51) and Japanese (Group I-C, n=11) controls were 0.11 ± 0.08 and 0.11 ± 0.03, respectively. The result shows that the ELISA system for detecting specific IgE antibodies has a low degree of cross-reactivity against intestinal helminthiasis widely spreading in Guatemala.

The microfilarial rates in the surveyed villages were 16.0% (El Regalo nominated as II-A), 40.6% (Santa Ines, II-B), 48.4% (Pacayalito, II-C) and 80.0% (Sibaja, II-D). The distribution of IgE values by age and sex of the inhabitants of each village (II-A–D) is shown in Fig. 2. The highest IgE value was observed in younger people (15–25 years old) of meso-endemic areas (II-B, C). In contrast, most inhabitants in the hyper-endemic area (II-D) had rather low IgE values. The IgE values decreased in proportion to age.

The IgE values in the young (below 35, average 23 ± 8 years old) and old (36 or more, average 48 ± 9 years old) subjects (mf density was less than 90/10 mm^2 skin-snip) were compared in order to examine whether the age affected the regulation of IgE antibody production in onchocerciasis. The IgE value was markedly low in the older age group (III-F) compared to that of the younger (III-E) ($P<$ 0.01) as indicated in Table 1. This parameter was found to be strongly influenced by sex. Old females (III-D) showed significantly lower IgE values than the young subjects (III-C) ($P<$ 0.05). Contrary to the females, there was no significant difference in the IgE values between the young (III-A) and old (III-B) males.

To examine the relation between mf density on the IgE values, the young (8–35 years old) subjects which consisted of 101 males and 93 females of 4 endemic villages were divided into five groups according to their mf density (Group IV-A, 0 per 10 mm^2 snip; IV-B, 1–10; IV-C, 11–30; IV-D, 31–90; IV-E, 91 or more). There was no significant difference in the

Table 1 Comparison of specific IgE values in the young and old subjects

	Young subjects				Old subjects			
Sex	No. of cases	Age in years	MFD*	IgE†	No. of cases	Age in years	MFD	IgE
M	48	23±7‡	25.8±24.6	0.28±0.22 (III-A)	33	47±8	28.0±25.4	0.23±0.11 (III-B)
F	31	24±8	22.6±22.6	0.36±0.23 (III-C)	11	53±11	26.6±26.7	0.20±0.15 (III-D)
M+F	79	23±8	24.6±23.8	0.31±0.23 (III-E)	44	48±9	27.7±25.4	0.22±0.12 (III-F)

*Number of microfilariae per 10 mm^2 of skin snip, †OD_{414}, ‡Mean ± SD,
III-A vs III-B, III-A vs III-C, III-B vs III-D: Not significant, III-C vs III-D: $P < 0.05$, III-E vs III-F: $P < 0.01$.

Table 2 Relationship between microfilarial density (MFD) and the specific IgE values (OD_{414}) in the young subjects (8–35 years old)

Group	IV-A	IV-B	IV-C	IB-D	IV-E
No. of cases	94	32	20	27	21
MFD*	0	1–10	11–30	31–90	91+
Mean OD_{414}	0.32	0.31	0.31	0.30	0.20
SD†	0.28	0.22	0.22	0.26	0.11

* Number of microfilariae per 10 mm^2 of skin-snip
† Standard deviation
The IgE values between IV-A vs IV-E, IV-B vs IV-E, IV-C vs IV-E are significant ($P < 0.05$).

average IgE values observed between the microfilarial positive (n=100, mean MFD, 0.29 ± 0.22) and negative (n=94, mean MFD, 0.32 ± 0.28) groups in endemic areas. As shown in Table 2, mean IgE value of group IV-E was significantly low ($P < 0.05$) in comparison with those of groups IV-A, B and C. There was a tendency that IgE response was suppressed with the rise in mf density in both sexes.

Discussion

The present study shows that the IgE antibody response of the onchocerciasis patients was depressed in those with higher microfilarial (mf) density. This phenomenon is quite interesting, although it is unclear whether the mf depressed the IgE antibody response and/or IgE antibodies could act as an effector and thus clear the mf from the skin like IgG antibodies. It has been reported that helminth infections suppressed specific and/or nonspecific IgE responses (Haig *et al.*, 1980; Ikeda and Fujita; 1980; Oikawa *et al.*, 1981), while the association of an elevated serum IgE level and the production of specific IgE antibodies in helminth infection (Johansson *et al.*, 1968; Jarrett and Miller, 1982) is well-documented. It is likely that the low IgE antibody level in the patients with heavy mf density has a protective effect from anaphylaxis (Altman and Levine, 1977). The present results are opponent to those of Kouemeni *et al.* (1982) who reported that the levels of IgE antibodies did not correlate with the level of mf density in African onchocerciasis. This discrepancy may be due to the differences in a size and/or genetic background of the studied populations or pathogenicity of the parasites distributing each in two continents. Recently, Titanji *et al.* (1985) reported total serum IgE and specific IgE levels in African onchocerciasis, using a radioallergosorbent test (RAST). They suggested that the "false" positive results for the RAST might be due to low levels of infection that were not detectable by the skin snip examination. Furthermore, Karam and Weiss (1985) reported that increasing concentrations of total serum IgE were found in younger children. Although the age classification of our study differs, higher IgE values seen in younger poeple will agree with their result.

Antibody dependent cell-mediated cytolysis has been recognized as a potent mechanism of the destruction of a variety of parasites (Capron *et al.*, 1982). The results of in vitro and in vivo experiments (Mehta *et al.*, 1980, 1982; Gusmão *et al.*, 1981; Ouaissi *et al.*, 1981) suggest that parasite-specific IgE antibodies play a role in protecting the host from filarial infection by interacting with effector cells. Studies by Kephart *et al.* (1984) have clearly shown the association between eosinophil degranulation, as evidenced by the deposition of major basic protein granule, and the killing of microfilariae of *O. volvulus* in vivo following the treatment

with diethylcarbamazine. The presence of Fc receptors specific for IgE was demonstrated on human eosinophils (Capron *et al.*, 1981). Furthermore, Kojima *et al.* (1985) have indicated that a nematode infection (*Nippostrongylus brasiliensis*-rat system) enhanced IgE-dependent eosinophil cytotoxicity to dinitrophenylated schistosomula. On this regard, the protective role of an interaction between IgE antibodies and eosinophils in human onchocerciasis should be investigated.

A decrease in the IgE antibody level of the aged group was observed. This coincides with the pattern of the total serum IgE and specific IgE antibody production in healthy population (Delespesse *et al.*, 1977). T lymphocytes are involved in the control of IgE antibody synthesis and a decline of their function is well established during ageing (Pisciotta *et al.*, 1967; Roberts-Thomson *et al.*, 1974; Weksler and Hutteroth, 1974).

Summary

The enzyme-linked immunosorbent assay (ELISA) for onchocerciasis was performed using a crude extract of adult *Onchocerca volvulus* as the antigen. Serum samples were obtained from inhabitants of both the endemic and nonendemic areas in Guatemala, and in Japan. The IgE-ELISA values of the majority of inhabitants were low in a hyper-endemic area, and decreased in proportion to their ageing. The microfilaria positives by the skin snip method were stratified according to their ages, sexes, and microfilarial densities. Then, a relationship between IgE-ELISA value and each factor was examined. The fifference in the mean IgE-ELISA value of males between young and old groups was less than that of female groups. Sex difference of IgE-ELISA value in the same age group was not found. Furthermore, mean IgE value of group with high microfilarial density was significantly low in comparison with that of group with low microfilarial density.

Acknowledgments

The authors thank Dr. H. A. Godoy B., Director of Servicio Nacional de Erradicacion de la Malaria, Ministerio de Salud Publica y Asistencia Social, Guatemala, and his staffs for their arrangement and cooperation in the field. We are also grateful to Dr. T. Abe of Miyazaki Medical College for his suggestions on the ELISA technique, to Drs. M. Sakamoto and T. Mimori for support throughout this study, and to Prof. T. Yoshimura of University of Occupational and Environmental Health, Japan, for his review of the manuscript.

References

1) Abe, T., Ishii, A. and Yoshihara, A. (1985): Application of a purified allergen from *Dermatophagoides pteronyssinus* for ELISA: Detection of specific IgE and IgG antibodies. Jpn. J. Allergol., 34, 284–288.
2) Akiyama, T., Ushijima, N., Anan, S., Nonaka, S., Yoshida, H. and Zea F., G. E. (1981): Immunological studies of onchocerciasis in Guatemala. I. Immunoglobulin levels in Guatemalan onchocerciasis. J. Dermatol., 8, 43–46.
3) Altman, R. and Levine, B. B. (1977): Effect of multiple injections of antigen on prolonged IgE antibody production in hypersensitive mice. J. Allergy Clin. Immunol., 59, 120–127.
4) Capron, A., Dessaint, J. P., Haque, A. and Capron, M. (1982): Antibody-dependent cell-mediated cytotoxicity against parasites. In Immunity and Concomitant Immunity in Infectious Diseases. Progress in Allergy 31, ed. by Kallós, P., Karger, Basel.
5) Capron, M., Capron, A., Dessaint, J-P., Torpier, G., Johansson, S. G. O. and Prin, L. (1981): Fc receptors for IgE on human and rat eosinophils. J. Immunol., 126, 2087–2092.
6) Delespesse, G., De Maubeuge, J., Kennes, B., Nicaise, R. and Govaerts, A. (1977): IgE mediated hypersensitivity in ageing. Clin. Allergy, 7, 155–160.
7) Gusmão, R. d'A., Stanley, A. M. and Ottesen, E. A. (1981): *Brugia pahangi*: Immunologic evaluation of the differential susceptibility to filarial infection in inbred Lewis rats. Exp. Parasitol., 52, 147–159.
8) Haig, D. M., Lima, G. C. and Mota, I. (1980): Antibody suppression in mice infected with *Nippostrongylus brasiliensis*. Parasite Immunol., 2, 175–187.
9) Ikeda, T. and Fujita, K. (1980): IgE in *Paragoniums ohirai*-infected rats: Relationship between titer, migration route, and parasite stage. J. Parasitol., 66, 197–204.
10) Jarrett, E. E. E. and Miller, H. R. P. (1982):

Production and activities of IgE in helminth infection. In Immunity and Concomitant Immunity in Infectious Diseases. Progress in Allergy 31, ed. by Kallós, P., Karger, Basel.

11) Johansson, S. G. O., Mellbin, T. and Vahlquist, B. (1968): Immunoglobulin levels in Ethiopian preschool children with special reference to high concentrations of immunoglobulin E (IgND). Lancet, i, 1118–1121.
12) Karam, M. and Weiss, N. (1985): Seroepidemiological investigations of onchocerciasis in a hyperendemic area of West Africa. Am. J. Trop. Med. Hyg., 34, 907–917.
13) Kawabata, M., Izui, S., Anan, S., Kondo, S., Fukumoto, S., Zea F., G. E. and Kobayakawa, T. (1983): Circulating immune complexes and their possible relevance to other immunological parameters in Guatemalan onchocerciasis. Int. Archs. Allergy appl. Immun., 72, 128–133.
14) Kephart, G. M., Gleich, G. J., Connor, D. H., Gibson, D. W. and Ackerman, S. J. (1984): Deposition of eosinophil granule major basic protein onto microfilariae of *Onchocerca volvulus* in the skin of patients treated with diethylcarbamazine. Lab. Invest., 50, 51–61.
15) Kojima, S., Yamamoto, N., Kanazawa, T., Shigematsu, H. and Ovary, Z. (1985): Enhancement of IgE-dependent eosinophil cytotoxicity to dinitrophenylated schistosomula by a nematode infection. Int. Archs Allergy appl. Immun., 76, 91–94.
16) Korenaga, M., Tada, I., Mimori, T., Sakamoto, M., Lujan, T. A., Zea F., G. E., Castro, J. C. and Yarzabal, L. (1983): Enzyme-linked immunosorbent assay (ELISA) in the detection of IgG antibodies in onchocerciasis using blood collected on fiterpaper. Jpn. J. Parasitol., 32, 347–355.
17) Kouemeni, L. E., Haque, A. and Capron, A. (1982): Detection of IgE antibodies in onchocerciasis. Possibility of using allergens from *Dipetalonema viteae* extracts that cross-react with allergenic determinants in crude extracts of *Onchocerca volvulus*. Clin. Exp. Immunol., 50, 541–548.
18) Mehta, K., Sindhu, R. K., Subrahmanyam, D. and Nelson, D. S. (1980): IgE-dependent adherence and cytotoxicity of rat spleen and peritoneal cells to *L. carinii* microfilariae. Clin. Exp. Immunol., 41, 107–114.
19) Mehta, K., Sindhu, R. K., Subramanyam, D., Hopper, K. and Nelson, D. S. (1982): IgE-dependent cellular adhesion and cytotoxicity to *Litomosoides carinii* microfilariae – nature of effector cells. Clin. Exp. Immunol., 48, 477–484.
20) Oikawa, Y., Ikeda, T. and Fujita, K. (1981): IgE antibody response in rats infected with *Litomosoides carinii*. Trop. Med., 23, 149–157.
21) Ouaissi, M. A., Haque, A. and Capron, A. (1981): *Depetalonema viteae*: Ultrastructural study on the in vitro interaction between rat macrophages and microfilariae in the presence of IgE antibody. Parasitology, 82, 55–62.
22) Ouaissi, A., des Moutis, J., Cornette, J., Pierce, R. and Capron, A. (1983): Detection of IgE antibodies in onchocerciasis using a semi-purified fraction from *Depetalonema viteae* total antigen. Int. Archs. Allergy appl. Immun., 70, 231–237.
23) Pisciotta, A. V., Westring, D. W., Deprey, C. and Walsh, B. (1967): Mitogenic effect of phytohaemagglutinin at different ages. Nature (London), 215, 193–194.
24) Roberts-Thomson, I. C., Wittingham, S., Yougchaiyud, U. and Mackay, I. R. (1974): Ageing, immune response and mortality. Lancet, ii, 368–370.
25) Somorin, A. O., Heiner, D. C. and Ajugwo, R. E. (1977): Immunoglobulin E in Nigerian onchocerciasis. Am. J. Trop. Med. Hyg., 26, 872–875.
26) Steward, M. W., Sisley, B., Mackenzie, C. D. and El Sheikh, H. (1982): Circulating antigen-antibody complexes in onchocerciasis. Clin. Exp. Immunol., 48, 17–24.
27) Tada, I., Korenaga, M., Mimori, T., Sakamoto, M., Yoshimura, T., Recinos C., M. M., Flores, O. F., Lujan T., A., Ochoa A., J. O., Castro, J. C. and Zea F., G. (1985): A comparative study of several diagnostic measures applied in Guatemalan onchocerciasis. Jpn. J. Parasitol., 34, 261–272.
28) Titanji, V. P. K., Monebenimp, F. and Ngu, J. L. (1985): Serum immunoglobulin E level in onchocerciasis: The development of a radioallergosorbent test for *Onchocerca volvulus* infection. Trop. Med. Parasit., 36, 12–16.
29) Weiss, N., Hussain, R. and Ottesen, E. A. (1982): IgE antibodies are more species-specific than IgG antibodies in human onchocerciasis and lymphatic filariasis. Immunology, 45, 129–137.
30) Weksler, M. E. and Hutteroth, T. H. (1974): Impaired lymphocyte function in ageing. J. Clin. Invest., 53, 99–104.

中米型オンコセルカ症：ELISA 法による特異的 IgE 抗体の検出

是永正敬[1)]　多田　功[1)]　橋口義久[2)]　高岡宏行[3)]　馬場　稔[3)]
JULIO C. CASTRO[4)]　GUILLERMO E.ZEA F.[4)]
([1)]熊本大学医学部寄生虫病学教室 [2)]高知医科大学寄生虫学教室 [3)]大分医科大学医動物学教室 [4)]グァテマラ共和国厚生省マラリア防圧部オンコセルカ部門)

Onchocerca volvulus 成虫抗原を用いた ELISA 法でオンコセルカ症患者血清中の IgE抗体を測定した．被検血清はグァテマラにおける同症流行地及び非流行地住民，また対照として寄生虫疾患のない日本人から得た．IgE-ELISA 値は仔虫保有率の高い地域ほど低く，また年齢と共に低下していた．そこで検皮法による仔虫陽性者を年齢別，性別，仔虫密度別に層別化して IgE-ELISA値との関連を調べた．男性では若年齢層と高年齢層の間で，女性の場合ほど大きな差はみられなかった．また同年齢層で男女の差はなかった．若年齢層を仔虫密度別に比較してみると，高仔虫密度保有者群において IgE-ELISA 値が有意に低下していた．