JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1988, p. 739–742
0095-1137/88/040739-04$02.00/0

Vol. 26, No. 4

Role of Specific Immunoglobulin E to Excretory-Secretory Antigen in Diagnosis and Prognosis of Hookworm Infection

NIRMAL KUMAR GANGULY,[1] RAMESH CHANDER MAHAJAN,[2]* RAKESH SEHGAL,[2] PRABHAKARA SHETTY,[3] AND JUNG BAHADUR DILAWARI[3]

Departments of Experimental Medicine,[1] Parasitology,[2] and Hepatology,[3] Postgraduate Institute of Medical Education and Research, Chandigarh 160012, India

Received 9 March 1987/Accepted 28 December 1987

Total immunoglobulin E (IgE) and specific IgE were assayed by a radioimmunosorbent test and a reverse enzyme immunoassay in patients with hookworm infections before and after treatment. A total of 77 subjects (30 patients with hookworm infections and 47 subjects as controls) were studied. Both specific and total IgE levels in serum and jejunal juice were raised in hookworm patients. There was a significant decrease in IgE levels after therapy. Total IgE levels were raised in other nematode infections, but specific IgE levels were low. The reverse enzyme immunoassay for specific IgE was highly specific (96%) and sensitive (100%) and may be used in the serodiagnosis of hookworm infections.

Hookworm infections caused by *Ancylostoma duodenale* and *Necator americanus* affect 25% of the world's population (3, 17). The magnitude of the infections can be judged from the fact that an estimated 7 million liters of blood per day is lost because of the parasites, causing a high degree of mortality and morbidity (16). A study of the pathophysiology of the parasite and the role of acquired immunity in the parasite life cycle is an important prerequisite for any attempt to prepare a vaccine effective in preventing the disease.

Routine examination of single stool specimens may be negative in more than half of the patients, even when concentration techniques are used. To obviate this drawback, various serological tests have been evaluated for diagnostic purposes. The skin test with an aqueous extract of the parasite was introduced as early as 1928 (4). Lobel et al. (14) evaluated the skin test with a larval antigen and concluded that, owing to the high frequency of false positivity (30% in nonendemic areas), the test was unsuitable for routine use. Various other serological tests, such as the complement fixation test and fluorescent-antibody test, have a high frequency of false positivity (30%), and no correlation between titers and worm load has been demonstrated (1).

A microenzyme-linked immunosorbent assay was recently evaluated for diagnostic purposes. The test had a sensitivity of 97% and a specificity of 92% with excretory-secretory antigens, indicating its value in the diagnosis of hookworm infections. The levels of immunoglobulin E (IgE) are also important in nematode infections. This fact has been observed in experimental infections with *Nippostrongylus braziliensis* and also in human hookworm infections (7, 9, 10, 13, 18). A marked elevation in the IgE response was seen, but its exact role in the pathogenesis of the disease is not clear. Its role in diagnosis and prognosis is also not clear, although a fall has been reported after drug treatment (7, 12, 13); these workers studied total IgE but not specific IgE against hookworm infections. We have studied the response of total IgE as well as specific IgE in serum as well as jejunal fluid from patients with hookworm infections. Furthermore, the local and systemic IgE levels were measured after specific treatment of patients to determine the prognostic value of IgE.

MATERIALS AND METHODS

Patient selection. Six groups of patients were chosen. They were of both sexes and ranged in age from 15 to 65 years.

(i) Group A. Group A consisted of 30 patients with hookworm infections confirmed by stool examination. For all patients multiple stool examinations were done by direct and concentration methods. Stool samples were also examined after 1 month of specific therapy for hookworm infections.

(ii) Group B. Group B consisted of 11 patients who had vague abdominal complaints and for whom three consecutive stool samples were negative by the direct and concentration methods.

(iii) Group C. Group C consisted of patients with proven hydatid disease diagnosed clinically, serologically, and surgically.

(iv) Group D. Group D consisted of five patients with ascaris infections confirmed by stool examination.

(v) Group E. Group E consisted of four patients with cysticercosis diagnosed clinically, serologically, and by biopsy.

(vi) Group F. Group F consisted of 17 normal healthy adults determined to be negative for hookworms and other parasites by multiple stool examinations by the concentration method. These patients did not have any history of suffering from any infection during the preceding 3 months.

Preparation of excretory-secretory antigen. The antigen was prepared by the method described by Katiyar et al. (11) with *A. duodenale* larvae. Third-stage infective larvae were thoroughly washed in normal saline and transferred to a conical flask containing normal saline; the flask was incubated at 37°C for 4 h in a water bath. The contents of the flask were spun at 2,000 rpm for 10 min (REMI centrifuge). The supernatant containing the excretory-secretory products of the larvae was pipetted out, filtered through a 0.22-μm-pore membrane filter (Millipore Corp.), and preserved until further use.

Collection of samples. Venous blood (10 ml) was collected from all test subjects and controls in the beginning of the study and 4 weeks after the start of drug treatment. Serum

* Corresponding author.

was separated and stored at −70°C until use. Jejunal juice was obtained only from groups A (30 patients) and B (11 patients) (6). Only 25 patients in group A were available for follow-up after treatment.

Treatment of hookworm-infected patients. Bephenium hydroxynaphthoate (5 g; containing 2.5 g of bephenium hydroxynaphthoate base) was administered orally on an empty stomach daily for 3 days. In addition, patients were also given ferrous sulfate (200 mg) three times daily for 3 weeks.

Estimation of total IgE. Total IgE was estimated by a radioimmunosorbent test with the PRIST kit (Pharmacia Diagnostics).

Estimation of specific IgE. A reverse enzyme immunoassay (REIA) was carried out by the method of Ignacio et al. (8) to measure specific IgE. Briefly, microenzyme-linked immunosorbent assay M129 plates (Dynatech Laboratories, Inc.) were coated for 16 h at 4°C with a 1/100 dilution of monospecific antihuman IgE in phosphate-buffered saline. The plates were washed four times with phosphate-buffered saline–Tween 20 and neutralized with 0.5% human serum albumin in phosphate-buffered saline for 6 h at room temperature. Human serum (0.05 ml) was added, and the plates were allowed to stand for 18 h at room temperature. After the plates were washed four times with phosphate-buffered saline–Tween 20, 0.1 ml of antigen conjugate (horseradish peroxidase; Sigma Chemical Co.) prepared according to the method of Murayama et al. (15) was added to each well, and the plates were incubated for 6 h. The plates were again washed, a substrate consisting of *ortho*-phenylenediamine and hydrogen peroxide was added, and the plates were placed in the dark. After 30 min, the reaction was stopped with 4 N sulfuric acid, and readings were taken on a microenzyme-linked immunosorbent assay reader (Dynatech) at 490 nm.

Statistical analysis. The Student *t* test (unpaired and paired) was used to analyze the results.

RESULTS

Serum IgE levels. Both total IgE and specific IgE levels were markedly elevated in hookworm patients (Fig. 1 and 2).

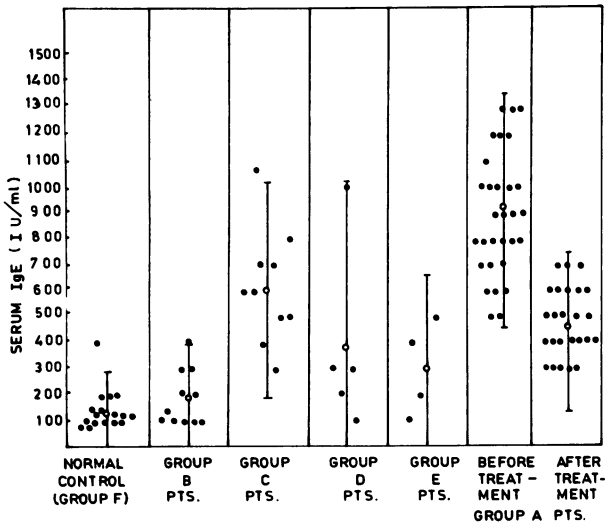

FIG. 1. Total serum IgE levels in different groups of patients (PTS.). Bars represent the mean ± 2 SD.

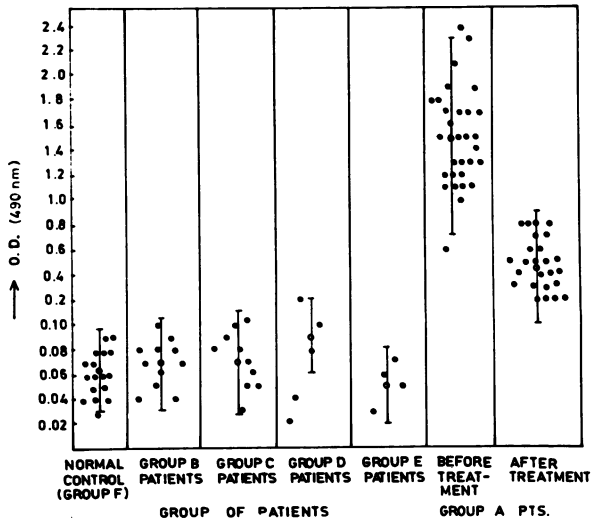

FIG. 2. Specific serum IgE levels in different groups of patients (PTS.) as measured by REIA with excretory-secretory antigen. The OD represents hookworm-specific IgE in 0.05 ml of undiluted serum. Bars represent the mean ± 2 SD.

The total serum IgE level was 913 ± 226 IU/ml before treatment; after therapy it decreased to 460 ± 141 IU/ml ($P < 0.001$). The level in the normal control group was 145 ± 80 IU/ml, indicating a highly significant rise in the infected group ($P < 0.001$). In group B patients the rise was not statistically significant, but patients with other nematode infections, cysticercosis, and hydatid disease had a significant rise in IgE levels (Fig. 1) ($P < 0.001$ and $P < 0.05$, respectively).

Specific serum IgE levels in infected patients were high when compared with levels in controls (optical densities, [OD], 1.510 ± 0.397 and 0.062 ± 0.018, respectively, in REIA) (Fig. 2). After therapy there was a significant decrease in the specific IgE levels (OD, 0.476 ± 0.205; $P < 0.001$). There was also a significant difference in the specific IgE levels in hookworm-infected patients (pretreatment OD, 1.510 ± 0.397) and patients infected with other nematodes (OD, 0.073 ± 0.027), group D patients (OD, 0.088 ± 0.070), and group E patients (OD, 0.052 ± 0.017).

Jejunal IgE levels. Total jejunal IgE levels were significantly increased in hookworm patients as compared with controls (310 ± 161 and 111 ± 61 IU/ml, respectively; $P < 0.001$); with treatment there was a significant decrease in these levels (145 ± 85 IU/ml; $P < 0.001$) (Fig. 3).

Specific jejunal juice IgE levels in infected patients were also significantly elevated when compared with levels in controls (OD, 0.893 ± 0.334 and 0.045 ± 0.057, respectively, $P < 0.001$). There was also a significant decrease in the specific IgE levels after therapy (OD, 0.448 ± 0.212; $P < 0.001$) (Fig. 4).

Specific IgE (serum and jejunal) levels were very low in normal controls (OD, 0.062 ± 0.018), parasitic controls (OD, 0.073 ± 0.027), and patients suffering from hydatid disease and cysticercosis (OD, 0.088 ± 0.07 and 0.052 ± 0.017, respectively).

DISCUSSION

Serological tests for the diagnosis of hookworm infections have never gained popularity because of the high frequency

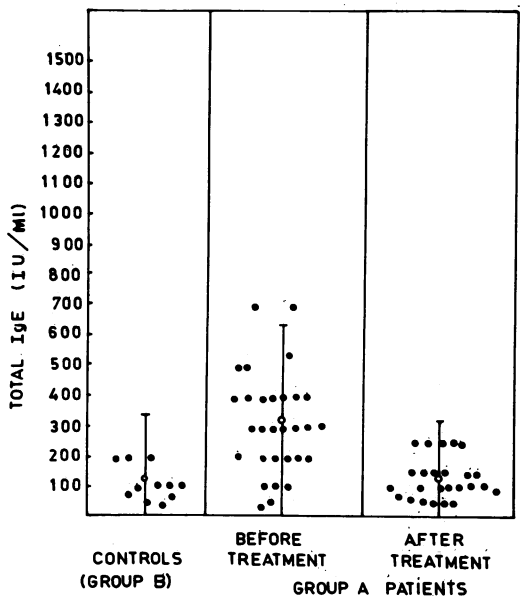

FIG. 3. Total jejunal fluid IgE levels in different groups of patients. Bars represent the mean ± 2 SD.

of false-positive results with most of these tests. Most of the work concerning immune responses after infection has been experimental, with only a few human studies. The human studies have also taken into consideration systemic responses, and local immune responses have rarely been evaluated (13).

In this work we studied the levels of total IgE antibodies before and after treatment of hookworm infections. In addition, we evaluated the levels of specific IgE antibodies directed against the excretory-secretory antigens of third-stage hookworm larvae. The emphasis was on the diagnostic as well as prognostic value of the determination of these antibody levels by the REIA.

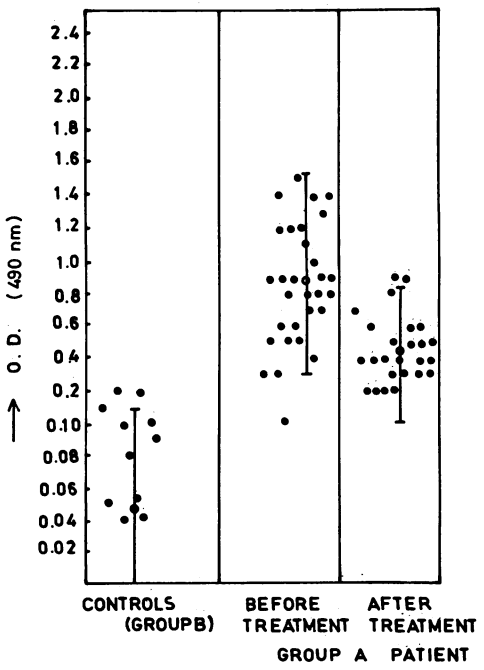

FIG. 4. Specific jejunal fluid IgE levels in different groups of patients as measured by REIA with excretory-secretory antigen. The OD represents hookworm-specific IgE in 0.05 ml of undiluted jejunal fluid. Bars represent the mean ± 2 SD.

The levels of serum IgE (total) were significantly elevated in hookworm patients, a result which is expected in most nematode infections. The titers (serum and jejunal) dropped after treatment, revealing the requirement of antigenic stimulation for the continued secretion of IgE. Similar findings have been reported previously (7, 12, 13). Jejunal IgE levels were also elevated, indicating the local stimulation and secretion of jejunal IgE.

Specific IgE levels were measured by REIA in both serum and jejunal juice (8). This is the first study of its kind measuring the specific IgE response to the excretory-secretory antigens of hookworm larvae. The levels of IgE measured by this test were elevated significanly in the serum and jejunal fluid of hookworm patients. The titers dropped significantly after specific treatment for hookworm infections. Considering these above two facts, we conclude that the test has diagnostic as well as prognostic potential. Specific IgE is elevated in serum in hookworm patients, and the titers drop quite soon after treatment, indicating that a past infection does not interfere in diagnosis and that a fall in the IgE levels represents successful therapy. In addition, with the mean ± 2 standard deviations (SD) as the cutoff value, the test shows 100% sensitivity and 96% specificity.

The role of various immunoglobulins, i.e., IgG, IgM, and IgA, in protection against hookworm and other nematode infections is still not clear. Local IgA may be protective. IgE may cause local anaphylaxis, leading to worm expulsion (2). The suppression of IgE has been shown to decrease the resistance of rats to *Trichinella spiralis* infections (5).

In conclusion, we recommend the estimation of specific IgE levels in hookworm patients because it seems to have promising diagnostic as well as prognostic applications and a very high sensitivity (100%) and specificity (96%).

LITERATURE CITED

1. **Ball, P. A. J., and A. Bartlett.** 1969. Serological reactions to infection with *Necator americanus*. Trans. R. Soc. Trop. Med. Hyg. **63**:362–369.
2. **Barth, E. E. E., W. F. H. Jarrett, and G. M. Urquahart.** 1966. Studies on mechanism of self cure reaction in rats infected with *Nippostrongylus braziliensis*. Immunology **10**:459–464.
3. **CCTA/WHO African Conference on Ancylostomiasis.** 1963. W.H.O. Tech. Rep. Ser. **255**:5–30.
4. **Coventry, F. A., and W. H. Taliaferro.** 1928. Hypersensitiveness to helminth proteins. 1. Cutaneous tests with proteins to ascaris, hookworm and trichuris in Honduras. J. Prev. Med. **4**:273–288.
5. **Desslin, A. J., W. L. Parker, S. L. James, and J. R. David.** 1981. IgE antibody and resistance to infection. 1. Selective suppression of IgE antibody response in rats diminishes the resistance and eosinophil response to *Trichinella spiralis* infection. J. Exp. Med. **153**:423–436.
6. **Dilawari, J. B., S. K. Sharma, and B. S. Anand.** 1980. Single intubation technique for the assessment of malabsorption and exocrine pancreatic function. Indian J. Med. Res. **72**:770–774.
7. **Grove, D. I., T. O. Burston, and I. J. Forbes.** 1974. Fall in IgG levels after treatment for hookworm. Clin. Exp. Immunol. **18**: 565–569.
8. **Ignacio, M., M. Cuevas, V. Urena, R. Alcover, and A. Bootello.** 1983. Reverse enzyme immunoassay for the determination of *Dermatophagoides pteronyssimus* IgE antibodies. Int. Arch. Allergy Appl. Immunol. **71**:285–287.
9. **Jarrett, E. E. E.** 1972. Potentiation of reagenic (IgE) antibody to

ovalbumin in rat following sequential trematode and nematode infection. Immunology **22**:1099–1101.
10. **Jarrett, E. E. E., D. M. Haig, and H. Bazin.** 1976. Time course studies on rat IgE production in *Nippostrongylus braziliensis* infection. Clin. Exp. Immunol. **24**:346–351.
11. **Katiyar, J. C., P. Govil, and A. B. Sen.** 1972. Immunological studies with *Nippostrongylus braziliensis*. A search for a functioning antigen in larval stages. Indian J. Exp. Biol. **10**:201–204.
12. **Kojima, S., M. Yokogawa, and T. Tada.** 1972. Raised levels of serum IgE in human helminthiasis. Am. J. Trop. Med. Hyg. **21**:913–918.
13. **Kumar, N., P. S. Gupta, and K. Saha.** 1980. Serum and intestinal immunoglobulins in patients of ancylostomiasis. Indian J. Med. Res. **71**:531–537.
14. **Lobel, H. O., I. G. Kagan, and R. L. Kaiser.** 1968. Evaluation of parasitologic and intradermal tests for detection of hookworm infection. Am. J. Epidemiol. **87**:58–72.
15. **Murayama, A., K. Shimada, and T. Yamamoto.** 1978. Modification of immunoglobulin G using specific activity of a sugar moiety. Immunochemistry **15**:523–528.
16. **Plorde, J. J.** 1983. Intestinal nematodes, p. 1225–1231. *In* R. G. Petersdorf and R. D. Adams (ed.), Harrison's principles of internal medicine, 10th ed. McGraw-Hill Book Co., New York.
17. **Schmidt, G. D., and L. S. Roberts.** 1985. Foundations of parasitology, 3rd ed., p. 1–6. C. V. Mosby Co., St. Louis.
18. **Sinski, E., and P. H. Holmes.** 1977. *Nippostrongylus braziliensis*: systemic and local IgA and IgG immunoglobulin responses in parasitized rats. Exp. Parasitol. **43**:382–389.