

SKIN TEST ANTIGENS FOR IMMEDIATE HYPERSENSITIVITY PREPARED FROM INFECTIVE LARVAE OF *STRONGYLOIDES STERCORALIS*

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Abstract. More rapid and simplified diagnostic procedures are needed for the diagnosis of strongyloidiasis. One approach is the use of an immediate hypersensitivity skin test that would reliably identify infected people. Accordingly, somatic and excretion/secretion (E/S) antigens were prepared from filariform larvae of *Strongyloides stercoralis* and were treated to remove possible adventitious agents. By use of a quantitative method for measurement of skin reactions, several preparations of the 2 antigens were tested in uninfected controls and in various groups of patients. Doses of 0.35 µg of E/S and 4 µg of somatic antigens elicited positive skin tests in 82–100% of infected people, depending on clinical status. A lower frequency of positive skin tests was found in strongyloidiasis patients also infected with human T-cell lymphotropic virus type 1. Cross-reactions, especially to somatic antigens, were frequently found in patients with filarial infections. Despite these limitations and the need for further study of specificity, these results provide a basis for future development of a diagnostic skin test antigen for strongyloidiasis.

INTRODUCTION

Difficulties in diagnosis of chronic infections caused by *Strongyloides stercoralis* occur because of the sporadic and scanty excretion of larvae.^{1–3} Enzyme-linked immunosorbent assay (ELISA) serology may not differentiate current from past infections, may cross-react with certain filarial infections, and is not always readily available.⁴ Therefore, rapid and simplified methods of diagnosis, such as an immediate hypersensitivity skin test for detection of current or recent infections, would be very useful.

In 1926, Fülleborn⁵ described immediate skin test reactions in patients with strongyloidiasis with extracts of dried and powdered larvae. In 1949, Brannon and Faust⁶ reported their experience with skin test reactions by use of an antigen prepared from larvae of *Strongyloides fulleborni*. Pellegrino and others⁷ in Brazil described specific immediate skin test reactions by use of antigens from *Strongyloides ratti*, and an immediate hypersensitivity skin test for strongyloidiasis with a *S. ratti* antigen was reported by Tribouley and colleagues⁸ from France. They used a disrupted and delipidized larval antigen at a protein concentration of 30 µg of N₂ per milliliter in a volume of 0.05 mL per dose. More recently, Japanese workers reported their experience with an immediate skin test with an antigen prepared from filariform larvae of *S. stercoralis*.⁹ In each of the instances cited above, as well as with the method described in this report, the antigen was prepared from charcoal cultures of larvae from fecal specimens of experimentally infected animals. Increasingly stringent administrative controls on biologicals have understandably inhibited further work in this country on reagents for parenteral injection prepared directly from organisms excreted in the feces.

Nevertheless, with the safeguards described below, we have accumulated experience with the use of experimental immediate skin test antigens prepared from infective larvae (L3) of *S. stercoralis*. This report describes the preparation of immediate hypersensitivity skin test antigens and summarizes results obtained with their use in > 150 people. Patients tested included 20 uninfected controls and 29 parasite-positive case patients to establish values for a positive test, 17 World War II ex-prisoners of war (POWs) with in-

fections of ≥ 40 years' duration, 18 patients with potentially cross-reactive filarial infections, and 67 patients studied in Brazil.¹⁰ Although it is unlikely that the relatively crude antigens described in this study will have widespread use as diagnostic reagents, our results provide insight into immunoglobulin (Ig) E-mediated responses in *Strongyloides* infections. Our results also illustrate the potential for a standardized and easily produced preparation, such as a recombinant antigen, for rapid and convenient diagnosis of *Strongyloides* infection.

MATERIALS AND METHODS

Patients. The protocol for this study which required written informed consent was approved and reviewed annually by a National Institutes of Health Institutional Review Board. Use of the skin test antigens was authorized and periodically reviewed as an Investigational New Drug by the Center for Biologics Evaluation and Research of the Food and Drug Administration. The 20 normal and presumed uninfected controls were mainly laboratory personnel with no detectable antibodies to *S. stercoralis* larval antigen.⁴ Four of the controls were World War II former POWs. The 29 infected subjects whose skin test reactions were used to establish a cutoff value for positive reactions were patients at the National Institutes of Health (NIH) Clinical Center with *S. stercoralis* larvae in fecal specimens ≤ 2 weeks before application of the skin tests.

A total of 18 patients with definite or probable filarial infections were studied. These included 9 people with *Loa loa*, 6 patients with *Onchocerca volvulus*, 1 with *Wuchereria bancrofti*, and 1 each with *Mansonella ozzardi* and *Mansonella perstans*. The diagnoses of these patients were established on the basis of a history of exposure in endemic areas, a compatible clinical picture, the presence of antifilarial antibodies, and, in 9 patients, by demonstration of microfilariae in the blood or the adult worm in the tissues.

The 17 ex-POWs had been incarcerated in Japanese prisoner-of-war camps in Southeast Asia during World War II.¹¹ *Strongyloides* larvae had been demonstrated in stool samples of some of the ex-POWs, but more detailed medical and laboratory records for this group were not available.

Preparation of antigens. Feces of infected *Patas* monkeys containing larvae of *S. stercoralis*, obtained originally from a Southeast Asian patient, were mixed with charcoal and water and cultured at room temperature for 7–16 days. This allowed a free-living generation to occur and development of larvae to the filariform stage. Filariform larvae were separated from the charcoal by the Baermann procedure.¹² Larvae were collected and repeatedly centrifuged at $1,250 \times g$ (4°C , 15 min) with RPMI cell culture medium containing penicillin and streptomycin (100 U or 100 $\mu\text{g}/\text{mL}$) for Antigen 1. Larvae for Antigen 2 were washed and treated with RPMI medium containing 50 $\mu\text{g}/\text{mL}$ of gentamicin. All larvae were subjected to a second Baermann procedure, concentrated by centrifugation, and then exposed to a 0.25% sodium hypochlorite solution (Clorox; The Clorox Company, Oakland, CA) for 3–4 min and washed 6 times by centrifugation at $150 \times g$ for 3 min at 4°C in 40 mL of RPMI medium containing antibiotics, as described above. Larvae thus treated were incubated in RPMI medium without serum at a concentration of 30×10^3 larvae/mL but with antibiotics at 33°C for 24 or 48 hr.

After incubation, the culture fluids containing larvae yielded either no growth or only 1–3 colonies of bacteria when plated on blood agar medium. The incubating fluids, after separation from larvae by centrifugation and Millipore filtration (0.45- μm pore size), were stored at -70°C . The larvae were stored frozen, then thawed and sonicated later. The disrupted larvae were centrifuged at $32,000 \times g$ for 30 min. The supernatant was then filtered (0.45- μm sterilizing filter), and the resulting material was referred to as the somatic antigen.

The incubation fluids were thawed, pooled, and lyophilized. The lyophilized material was reconstituted in a 10-fold less than original volume of phosphate-buffered saline at pH 7.2 and filtered (0.45- μm sterilizing filter). This fraction was termed the excretion/secretion (E/S), or metabolic, antigen. Antigens were stored at -70°C until treatment with 1:4,000 formalin.

Next, we thawed the E/S or somatic antigens, added formalin to a final concentration of 1:4,000, and held them at $36\text{--}37^\circ\text{C}$ for 14 days. This treatment is similar to that used in preparation of inactivated poliomyelitis vaccine, the rationale being to inactivate enteric viruses that might have been present in the original fecal specimens containing the larvae. Phosphate-buffered saline was also incubated with 1:4,000 formalin for 14 days at $36\text{--}37^\circ\text{C}$ for use as a control antigen. Protein concentrations of the antigens after treatment with formalin were 800 $\mu\text{g}/\text{mL}$ for the somatic and 30 $\mu\text{g}/\text{mL}$ for the E/S antigen. Tests for safety included sterility, Limulus amoebocyte lysate assay for endotoxin, and inoculation into cell cultures for possible cytopathic agents and nonspecific toxicity. Phenol in a final concentration of 0.4% was added to the antigens as a preservative before distribution in sterile vials of 1 and 2 mL. Antigens were stored thereafter at $4\text{--}5^\circ\text{C}$.

Application, reading, and measurement of skin tests. Antigens in doses of 0.012 to 4.0 μg of protein were injected intradermally into the volar forearm in volumes of 0.1 mL with a 1.0-mL allergy syringe with an attached 27-gauge needle. The somatic and E/S antigens were used most commonly at protein concentrations of 40 μg and 3.5 $\mu\text{g}/\text{mL}$,

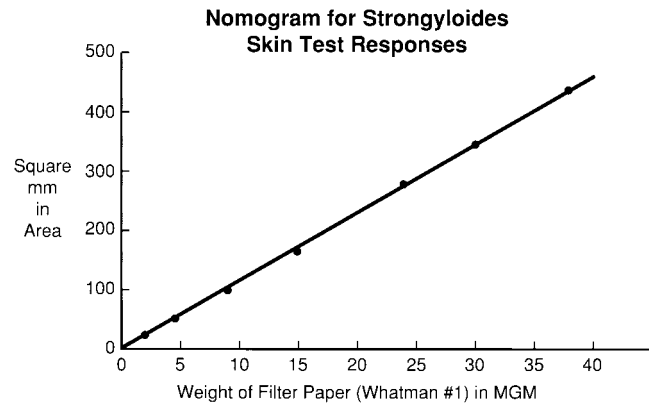


FIGURE 1. Nomogram used to convert weights of outlined skin test reactions on dried filter paper to area of reactions, measured in square millimeters.

respectively. These preparations, along with a control (diluent), were injected in a linear alignment 2–3 cm apart. Thus, the total amount of protein antigen injected into the skin did not exceed 4 μg . The wheals that developed in positive tests were easily discernible and reached their maximum size within 15 min. All of the wheals were outlined by marking on the skin with an indelible pen, and the reaction outlines were blotted or transferred onto a strip of moistened No. 1 Whatman filter paper.

After the paper had dried thoroughly, the reactions were cut out and weighed on an analytical balance to the nearest 0.1 mg. The weights of the reactions were converted to square millimeters of area by reference to a nomogram plotted from the weights of dried filter paper of known areas. A sample nomogram is shown in Figure 1. The area of the control site was subtracted from the area of reaction to the somatic and E/S antigens to obtain the final or net reaction size in mm^2 .

RESULTS

Criteria for positive skin test reaction. Twenty laboratory personnel, found to be presumably uninfected on the basis of negative ELISA serology and a negative stool examination for parasites, constituted the control group. The infected group comprised 29 people in whom *S. stercoralis* larvae had been demonstrated by fecal examination within the month before they were skin tested. These people had no known underlying medical condition that might influence the outcome of the test. The area of skin reactions to both antigens was recorded in rank order for the control and infected groups and plotted as to relative frequency of occurrence, as shown in Figure 2. Doses of somatic and E/S antigens used were 4.0 and 0.3 μg protein, respectively.

In the control group, 45–65% of net reactions (test minus control) were $\leq 1 \text{ mm}^2$, with a maximum reaction of 40 mm^2 . In the *Strongyloides*-infected people, the minimum reactions were 64 and 89 mm^2 to E/S and somatic antigens, respectively. In view of the large difference in size between the largest reaction in a control and the smallest reaction in an infected patient, any value between these extremes could be selected as a cutoff for positive reactions. A value of $\geq$

Cumulative Frequency of Reaction Sizes to *S. stercoralis*
Somatic and E/S Skin Test Antigens in Controls and Infected Individuals

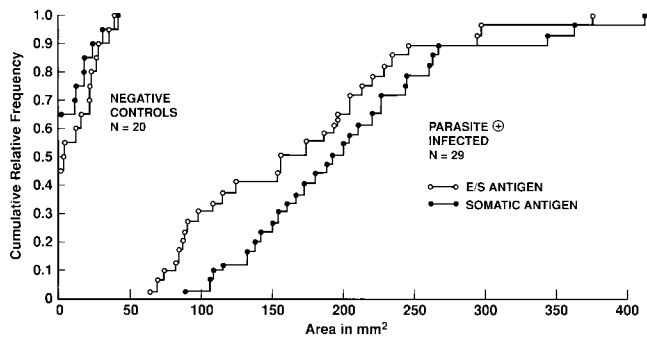


FIGURE 2. Cumulative frequency of reaction sizes to *Strongyloides stercoralis* somatic and excretion/secretion (E/S) skin test antigen preparation 1 in 20 negative controls and 29 people infected with *S. stercoralis*. Doses of somatic and E/S antigens used were 4.0 and 0.3 μg protein, respectively.

40 mm^2 was selected as a positive reaction for both the somatic and E/S antigens.

Dose-response of skin test reactions to antigens. Figure 3 shows the ranges and means of skin reactions when applied at 5-fold dilutions in doses varying from 0.012 to 4.0 μg of protein of the E/S and somatic antigens. The doses of

Dose Response to *S. stercoralis*
Skin Test Antigen (#1)

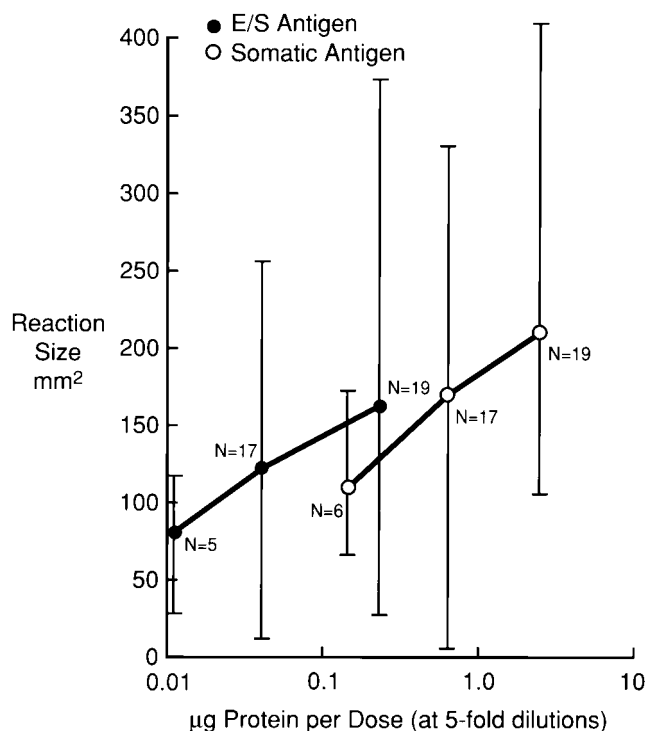
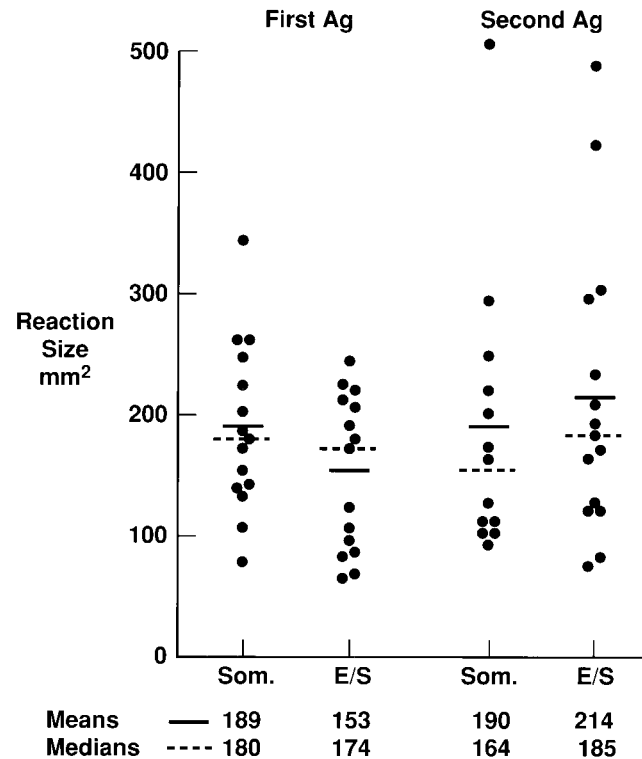


FIGURE 3. Ranges and means of skin test responses to somatic and excretion/secretion (E/S) antigen preparation 1, each given in 5-fold dilutions to the same person. The calculated protein concentration of somatic antigen actually injected per dose varied from 0.012 to 4.0 μg ; for the E/S antigen, doses injected varied from 0.06 to 0.3 μg .

Reactions in Same Individuals
Tested with First and Second
S. stercoralis Skin Test Antigens*



* 40 $\mu\text{g}/\text{mL}$ protein in somatic, and 3-3.5 $\mu\text{g}/\text{mL}$ in E/S antigens

FIGURE 4. Sizes of skin test reactions elicited with somatic and excretion/secretion (E/S) antigen (Ag) preparations 1 and 2 when tested at the same time in the same people. The ranges, means, and medians are shown.

antigen represent the calculated amount injected intradermally in a volume of 0.1 mL. Although there was a wide range in responses in the 17 or 19 people receiving the largest doses, the mean responses were linear with respect to antigen concentrations. Responses to E/S antigen were more sensitive, with protein antigen doses of 0.06 and 0.3 μg producing reactions equivalent to 0.16 and 0.8 μg , respectively, of somatic antigen.

Comparison of 2 separate batches of antigens. Two separate batches of skin test antigen (Antigens 1 and 2) were prepared several years apart, each consisting of a somatic and an E/S component. The only difference between the 2 preparations was that different antibiotics were used: penicillin and streptomycin in Antigen 1 and gentamicin in Antigen 2. Accordingly, it was of interest to compare the reactivity of the 2 batches for consistency in the same people at the same time. Figure 4 shows the ranges, means, and medians of the skin test reactions obtained in 15 people who were tested with both batches of antigen at the same time. Two of the 15 patients were not tested with somatic Antigen 2. Larvae of *S. stercoralis* were found in the stools of all patients who were skin tested.

Most of the patients (11 of 15) who received skin tests with Antigens 1 and 2 were refugees from Southeast Asia

TABLE 1

Summary of skin tests, fecal examinations, and antibody levels in 17 ex-prisoners of war

Patient no.	Skin test (mm ²)		Fecal exam for larvae*	Antibody titer†
	Somatic	Excretion/secretion		
1	174	129	+	<8
2	103	80	+	<8
3	220	139	+	32
4	208	139	+	32
5	243	173	+	64
6	138	81	+	64
7	171	115	+	128
8	11	<1	—	128
9	184	128	—	128
10	127	139	—	128
11	104	117	+	512
12	151	127	+	512
13	163	80	+	1,024
14	127	80	—	1,024
15	<1	<1	+	1,024
16	139	115	—	2,048
17	115	12	—	>4,096

* *Strongyloides stercoralis* larvae demonstrated 1–4 years before skin tests and bleedings for serology were performed. + = positive; — = negative.

† Reciprocal of serum dilution by enzyme-linked immunosorbent assay.

who had been in the United States for at least 2 years. None had acute signs or symptoms of strongyloidiasis, but a history of intermittent epigastric pain, episodic diarrhea, and recurrent urticaria over the lower abdomen, thighs, and buttocks could often be elicited.¹³ Most of the patients also had elevated peripheral blood eosinophilia.

Except for a few outlier reactions, especially to antigen 2, skin test responses to both antigens ranged between 60–300 mm². On the basis of the Wilcoxon rank test, the mean values for E/S skin test reactions for Antigens 1 and 2 were not statistically different ($P = 0.25$). A similar analysis of the mean values for the distribution of reactions to somatic Antigens 1 and 2 were found to be the same ($P = 0.49$).

Skin test responses in World War II ex-POWs. One group of *Strongyloides* patients that dramatically illustrates the long-term persistence of this infection are the ex-POWs who became infected in Japanese prison camps in Southeast Asia during World War II.^{14,15} In view of the presumed long duration of infection in these people (~ 40 years), their skin test responses were of particular interest because so many of them were positive. The results of fecal examinations for *S. stercoralis* larvae, tests for IgG antibodies to the parasite, and intradermal reactions to somatic and E/S skin test antigens for 17 of the ex-POWs are summarized in Table 1. Two of the 17 ex-POWs had negative skin tests to both antigens, and another responded only to the somatic antigen. Most of these ex-POWs had been treated with thiabendazole months to several years before the skin tests and bleedings for serology were performed.

Skin test reactions in patients with filarial infections. Because cross-reacting serum IgG responses to *S. stercoralis* somatic antigen have been noted in patients with filarial infections,⁴ immediate hypersensitivity responses to the *Strongyloides* antigens in some of these patients were also examined. The preponderance of patients with *Loa loa* and *Onchocerca* infections reflect the patient population referred to the NIH Clinical Center facility. Five of the 6 patients

TABLE 2

Reactions to *Strongyloides stercoralis* skin test antigens in patients with filarial infections

Skin test response*	Number of people in category indicated					
	On-chocerca	On-chocerca plus Loa	Loa	Wuchereria bancrofti	Other	Total
Positive somatic, positive E/S	3	2	1	—	—	6
Positive somatic, negative E/S	—	—	4†	—	2	6
Negative somatic, negative E/S	1	—	4	1	—	6
Total	4	2	9	1	2	18

* E/S = excretion/secretion.

† One of these patients was positive to excretion/secretion but negative to somatic antigen.

with positive reactions (≥ 40 mm²) to both somatic and E/S antigens had onchocerciasis, and 2 of the 6 were also considered to have *Loa loa*. Only 1 of the 6 patients with negative reactions to both antigens (< 35 mm²) had *Onchocerca* infection. Of the 4 negative reactors with loiasis, 2 were microfilaremic. The one remaining patient with negative reactions to both antigens had *W. bancrofti* in the biopsy of a skin lesion.

In 5 of the 6 instances in which positive skin tests were elicited in filarial patients with only 1 of the 2 *S. stercoralis* antigens, the positive reactions occurred with somatic instead of the E/S antigen. Similar results were found in patients with *M. ozzardi* and *M. perstans* infections. These results are summarized in Table 2.

Skin test responses in *Strongyloides* patients also infected with human T-cell lymphotropic virus type 1 (HTLV-1). Seven patients infected with HTLV-1 and *Strongyloides* exhibited a higher frequency of negative reactions than expected. Three of the 7 were negative to both the somatic and E/S antigens, and a fourth patient reacted only to the E/S antigen. There was no obvious association of either positive or negative responses with clinical expression of the HTLV-1 infection. The negative reactors included an asymptomatic carrier as well as a patient with tropical spastic paraparesis and one with T-cell leukemia. In a larger cohort of patients in whom this association was examined, nearly 40% of people infected with both *S. stercoralis* and HTLV-1 were negative by skin test, compared with $< 10\%$ in *Strongyloides* patients without HTLV-1. Further details are presented in another report.¹⁰

DISCUSSION

Except for the original brief report by Fülleborn⁵ in 1926 and the most recent article by Sato and others,⁹ the parasites used for preparation of skin test antigens in the past have been species of *Strongyloides* generally not infecting humans. In data not shown here, we prepared somatic and E/S skin test antigens from *S. ratti* and found many examples of discordant results when compared with the *S. stercoralis* antigens. This was especially striking with regard to the E/S antigens. One notable feature of our investigation of *Strongyloides* skin test antigens that warrants emphasis is that it is the first time an E/S or metabolic antigen has been evaluated for this purpose. All other skin test antigens described for this parasite, including the somatic antigen of this report,

have basically consisted of the soluble portion of disrupted larvae. Fewer cross-reactions to E/S antigen by patients with filarial infections suggest greater specificity of the E/S antigen. The protein concentration required to elicit a positive reaction with E/S antigen was ~ 3 -fold less than that required for the somatic antigen. Therefore, the antigen or antigens present in the E/S material would probably be the most specific as well as most sensitive for skin test purposes.

Critical comparison of results obtained by previous investigators with our results is difficult because of differences in the treatment of larvae, the diluents used, and the actual volumes of antigen delivered intradermally. The concentration of antigen used in most of the previous reports⁶⁻⁸ was expressed as 4–40 μg of nitrogen per milliliter, and the volumes of antigen injected varied between 0.02–0.10 mL. For the trial in Okinawa, the optimum antigen concentration was found to be 80 $\mu\text{g}/\text{mL}$ protein,⁹ or 1.6 μg protein per dose compared with the 4 μg of protein per dose of our somatic antigen; however, an intradermal injection volume as small as 0.02 mL would be difficult to deliver as reproducibly as a larger volume.

In the previously reported trials, the number of parasite-positive patients tested varied from as few as 8 to 148; yet it is interesting that, except for the initial study of Fülleborn,⁵ in which 7 of 10 infected patients had strongly positive reactions, the other studies showed surprisingly consistent positive reactions. Twenty-five to 52 parasitologically confirmed patients were skin tested in the 4 remaining studies before ours, and positive reactions were reported in 83–92%. In our initial cohort tested to establish a cutoff value, all 29 subjects were positive to both the somatic and E/S antigens. Several factors, including the immunologic background of the subjects tested, must be considered in interpreting the sensitivity of the skin test. For example, in the group of 17 ex-POWs with > 40 years' duration of infection, 88% had positive reactions to the somatic antigen, whereas the E/S antigen gave positive reactions in 82%. As reported elsewhere,¹⁰ when patients infected with HTLV-1 retrovirus are included, the percentage of positive reactions is significantly reduced.

When large numbers of presumably negative subjects are included in tests, the interpretation of specificity of the skin test becomes increasingly difficult. For example, in the report of Tribouley and others,⁸ when 1,367 people in an area endemic for intestinal nematodes were skin tested, some of the positive responses were ascribed to cross-reactions from pinworm, hookworm, or *Ascaris* infections; however, the presence of concomitant *S. stercoralis* infection could not be ruled out. Because the efficacy of diagnosis of chronic *Strongyloides* infection by stool examination is notoriously poor, even one or two careful fecal exams will not detect all infections. Therefore, a positive result in a putative negative control may actually be a truly positive test. For this reason, the emphasis at this stage of evaluating the use of a skin test for strongyloidiasis was on sensitivity of the test in eliciting positive responses in people with parasitologically documented infections. Obviously, much more needs to be done to establish specificity of immediate skin test reactions in people with other intestinal helminth infections such as hookworm and ascaris, but in whom cryptic *Strongyloides* infection can be ruled out.

Another consideration as to the clinical utility of the im-

mediate skin test is the persistence of a positive reaction after successful treatment. Here again, it may be difficult to prove that the infection is no longer present. Although IgG and IgG4 antibody levels to parasite antigen may decline significantly—even becoming undetectable by 6 months after treatment in some patients—in other patients, these antibodies remain elevated for up to 1.5 years (Neva FA and others, unpublished data). Anecdotal evidence suggests that the immediate sensitivity skin test to *Strongyloides* antigens can persist for several months after presumed effective treatment, but this has not been studied in a systematic fashion (Neva FA, unpublished data).

We are actively pursuing the possible use of a recombinant antigen for skin testing; however, it cannot be assumed that a recombinant antigen that can detect IgG and even IgE antibodies by ELISA can automatically also be capable of eliciting IgE-mediated immediate skin test reactions. To do so requires that the antigen bridge or cross-link 2 sites on the IgE antibody molecule on mast cells, causing discharge of vascular mediators that result in a wheal. In fact, we have found that only 1 of 4 recombinant *Strongyloides* antigens that were reactive with IgG by ELISA was able to release histamine *in vitro* from peripheral blood basophils of patients with strongyloidiasis (Neva FA and others, unpublished data). Nevertheless, the development of a suitably engineered recombinant skin test antigen for diagnosis of strongyloidiasis remains a challenging but worthwhile effort.

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REFERENCES

1. Neva FA, 1986. Biology and immunology of human strongyloidiasis. *J Infect Dis* 153: 397–406.
2. Grove DI, 1989. Diagnosis. Grove DI, ed. *Strongyloidiasis: A Major Roundworm Infection of Man*. Philadelphia: Taylor and Francis, 175–177.
3. Kaminsky RG, 1993. Evaluation of three methods for laboratory diagnosis of *Strongyloides stercoralis* infection. *J Parasitol* 79: 277–280.
4. Gam AA, Neva FA, Krotoski WA, 1987. Comparative sensitivity and specificity of ELISA and IHA for serodiagnosis of strongyloidiasis with larval antigens. *Am J Trop Med Hyg* 37: 157–161.
5. Fülleborn F, 1926. Spezifische kutanreaktionen bei infektion mit

- Strongyloides* und anderen helminthen. *Archiv Schiffs Trop Hyg* 30: 732–749.
6. Brannon MJC, Faust EC, 1949. Preparation and testing of a specific antigen for diagnosis of human strongyloidiasis. *Am J Trop Med Hyg* 29: 229–239.
 7. Pellegrino J, Chaia G, Pompeu JMM, 1961. Observações sobre a reação intradérmica com antígeno de *Strongyloides ratti* em pacientes com estrongiloidose. *Rev Inst Med Trop São Paulo* 3: 181–185.
 8. Tribouley-Duret J, Tribouley J, Pautrizel R, 1976. Intérêt des tests d'allergie cutanée pour le diagnostic de la strongyloidose. *Bull Soc Pathol Exot* 69: 360–367.
 9. Sato Y, Otsuru M, Takara M, Shiroma Y, 1986. Intradermal reactions in strongyloidiasis. *Int J Parasitol* 16: 87–91.
 10. Porto AF, Oliveira Filho J, Neva FA, Orge G, Alcantara L, Gam AA, Carvalho EM, 2000. Influence of human T-cell lymphocytotropic virus type 1 infection on serologic and skin tests for strongyloidiasis. *Am J Trop Med Hyg*. In press.
 11. Pelletier LL, Baker DB, Gam AA, Nutman TB, Neva FA, 1988. Diagnosis and evaluation of treatment of chronic strongyloidiasis in ex-prisoners of war. *J Infect Dis* 157: 573–576.
 12. Pereira Lima J, Delgado PG, 1961. Diagnosis of strongyloidiasis: importance of Baermann's method. *Am J Dig Dis* 6: 899–904.
 13. Gann PH, Neva FA, Gam AA, 1994. A randomized trial of single- and two-dose ivermectin versus thiabendazole for treatment of strongyloidiasis. *J Infect Dis* 169: 1076–1079.
 14. Gill GV, Bell DR, 1979. *Strongyloides stercoralis* infection in former Far East prisoners of war. *Br Med J* 279: 572–574.
 15. Grove DI, 1980. Strongyloidiasis in Allied ex-prisoners of war in south-east Asia. *Br Med J* 280: 598–601.