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Gelatin Particle Indirect Agglutination Test for Serodiagnosis of Human Strongyloidiasis

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Abstract

The indirect agglutination test with newly developed gelatin particles was tried to determine if it would be used for the serodiagnosis of human strongyloidiasis. The results were quite comparable to those of the usual indirect hemagglutination test and enzyme-linked immunosorbent assay. The gelatin particles possess many advantages as an antigen carrier, e.g. in handling, reading of the resulting pattern and preservation for a long period. The test is simple and rapid to perform without specialized equipment and it should be useful for mass screening for human strongyloidiasis.

Key words: Strongyloidiasis; indirect agglutination test; serodiagnosis; *S. stercoralis*

Introduction

Strongyloidiasis is a human intestinal parasitic disease caused by *Strongyloides stercoralis* infection. The disease has long been endemic in Okinawa prefecture, Japan, a district where other parasitic diseases have been almost completely eradicated in recent years. One of the unique properties of the parasite is the ability to propagate in its host by internal autoinfection. Due to the autoinfection, the continuous recycling of the parasite occurs in the host and it allows the long-term persistence of the parasitic infection. It is well documented that under the conditions of depressed immune competence, the infection produces a massive and often fatal systemic invasion by larval parasite as a consequence of increasing autoinfection (Scowden *et al.*, 1978).

In the previous studies, the authors have indicated several important problems of strongyloidiasis in Okinawa (Sato, 1986). One of the problems is that the detection of the parasite is frequently difficult in a long-standing, chronic infection. On the other hand, it has been demonstrated seroepidemiologically that patients with chronic strongyloidiasis frequently have an adult T-cell leukemia (ATL) viral infection in Okinawa (Nakada *et al.*, 1984; Fujita *et al.*, 1985; Sato & Shiroma, 1989). ATL is characterized by severe deficiencies in immune responsiveness, so that it appears that many patients with strongyloidiasis are at risk for massive infection in Okinawa. Therefore, the exact diagnosis by repeated fecal examination or by other reliable immunodiagnostic methods is essentially important to prevent such a severe infection.

In the present study, the authors tried to diagnose strongyloidiasis by the indirect agglutination test with a newly developed carrier particle.

Materials and Methods

Subjects

A total of ninety-two patients studied were diagnosed as strongyloidiasis by fecal examinations which were repeated for three consecutive days in a combination of the fecal concentration (a formalin-ether concentration method) and the fecal culture (Harada-Mori filter paper strip culture method). Sixty uninfected persons from Niigata prefecture, a non-endemic area for strongyloidiasis, served as control. Any of the

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subjects had no concurrent infection with other gastrointestinal parasite.

The sera of the patients with various helminth infections other than strongyloidiasis were also tested to determine whether there might be cross reactivity to the helminth infections. These cases were five anisakiasis, two hookworm disease, three angiostrongyliasis, four cysticercosis, a sparganosis, a unilocular echinococcosis, five clonorchiasis sinensis, a schistosomiasis mansoni and a case of *Rhabditis* infection.

Antigen preparation

Filariform larvae of *S. stercoralis* were obtained from feces of patients with relatively severe strongyloidiasis and the antigens were extracted from the larvae. The methods for fecal mass-culture to obtain large numbers of filariform larvae and that for antigen preparation from the larvae were the same as detailed elsewhere (Sato *et al.*, 1983; 1985). The protein concentration was adjusted to 50 μg/ml with 0.15 M phosphate buffer, pH 6.4 for the indirect agglutination test and to 10 μg/ml with 0.05 M carbonate buffer, pH 9.6 for the enzyme-linked immunosorbent assay (ELISA).

Indirect agglutination test

The test was performed with artificial gelatin particles (Fujirebio Inc., Tokyo) coated with *S. stercoralis* antigens. The technique for the gelatin particle agglutination test (GPAT) was almost the same as that of the indirect hemagglutination test (IHAT) by Boyden (1951), in which sheep red blood cells (SRBC) were used as the antigen carrier. The gelatin particles were washed four times by centrifugation with an excess of PBS and were adjusted to a 3% suspension. The suspension was mixed with an equal volume of 10^{-5} tannic acid (Merck: Darmstadt) dissolved in PBS. The tannin treatment was allowed to remain in an ice bath for 15 min. During the tannin treatment, the mixture was well stirred every 5 min. The tanned particles were washed three times with a cold PBS and were resuspended in the initial volume of physiological saline. The suspension was then poured into a tube containing an equal volume of antigen solution. The mixture was kept 15 min in a water bath at 37°C and shaken every 5 min. The particles were washed four times with PBS containing 0.6% inactivated normal rabbit serum (NRS) and were suspended finally to make a 1% suspension.

For estimation of agglutination titers, a microtiter technique using a plastic microplate with U-bottomed wells was utilized. A drop (25 μl) of the serum diluent (0.6% NRS-PBS) was placed into each well using a caribrated pipette dropper, and then the test serum was diluted in the wells in a serial 2-fold dilution using a diluter. A drop of the antigen-particle suspension was further added to each well and the plate was shaken to suspend the particles. The particles were then allowed to settle for 3 hr and the settling patterns at the bottoms were read. The negative and positive agglutination patterns were interpreted as follows: particles concentrated in the shape of a compact button in the center of the well (−); particles spread out uniformly covering the bottom of the well (+). The antibody titers were determined as the highest serum dilution giving a positive pattern.

The usual IHAT with SRBC was also performed to compare the results. The methods for sensitization of SRBC with the antigens and for estimation of antibody titers were the same as above.

Enzyme-linked immunosorbent assay

The ELISA was also applied for assessment of serum antibodies to *S. stercoralis*. The conventional technique for micro-ELISA using a microtiter plate was described fully in a previous paper (Sato *et al.*, 1985).

Statistics

Association between various parameters were evaluated using Spearman's coefficient of rank correlation. A P value of more than 0.05 was considered not significant (NS).

Results

The agglutination patterns determined with the gelatin particle and SRBC carriers for nine patients and three uninfected controls were com-

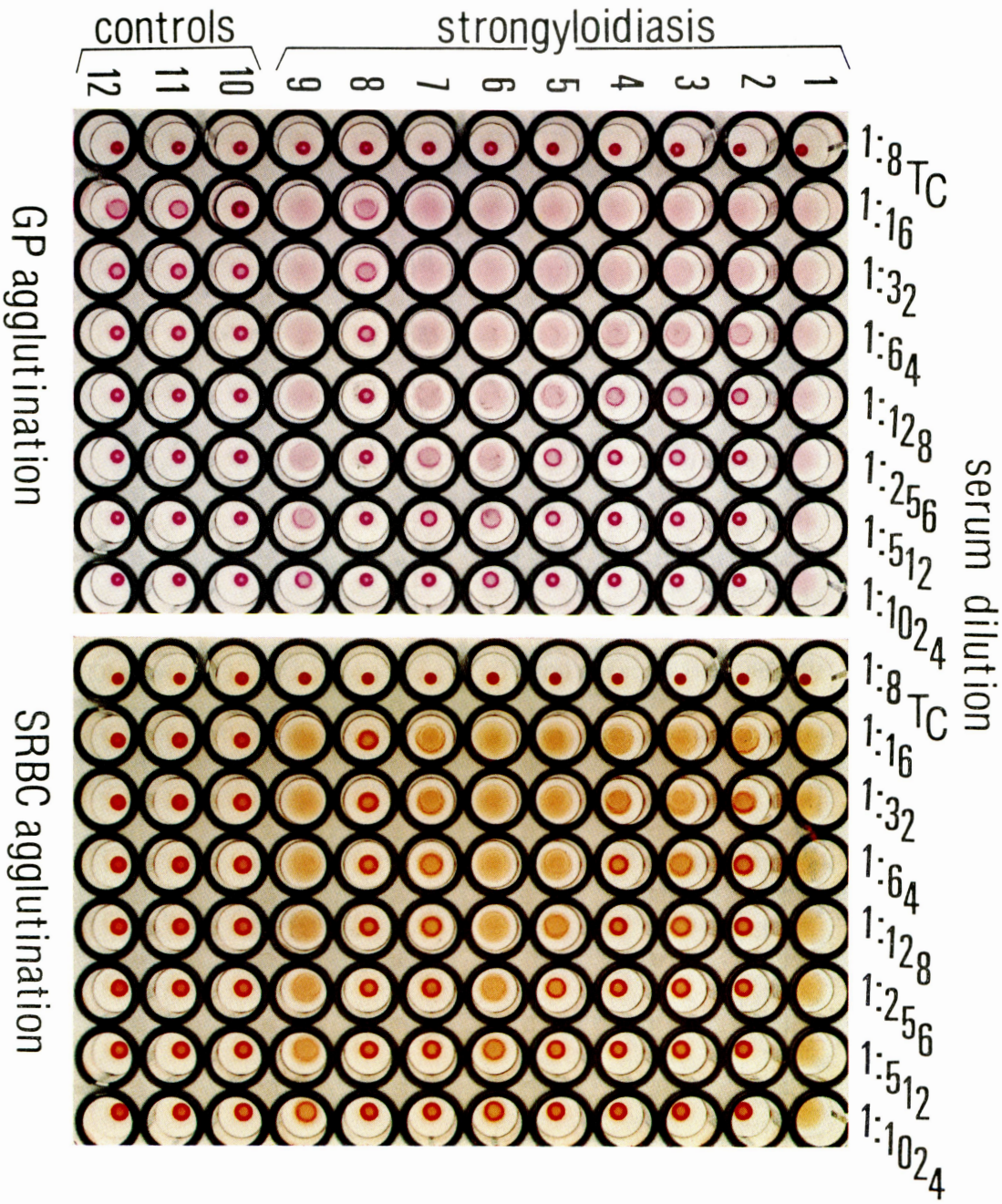

Fig. 1 Agglutination patterns of the gelatin particles (GP) and sheep red blood cells (SRBC) sensitized with *S. stercoralis* antigens in 9 patients with strongyloidiasis (Case No. 1-9) and 3 uninfected controls (Case No. 10-12). The antigen-carriers were mixed with the serially diluted sera and the resulting agglutination patterns were compared as shown in the left (GP agglutination) and right (SRBC agglutination) figures. Tc: tannin controls with nonsensitized carrier particles.

pared in Fig. 1. Positive agglutination patterns were observed in all of the patients but not in the controls. The resulting patterns of gelatin particles were similar to those of SRBC carrier in each patients.

Fig. 2 represents the relation of the agglutination titers estimated by the GPAT and the IHAT for 25 patients with strongyloidiasis. Although the antibody titers assessed by the GPAT were generally higher than those assessed by the IHAT,

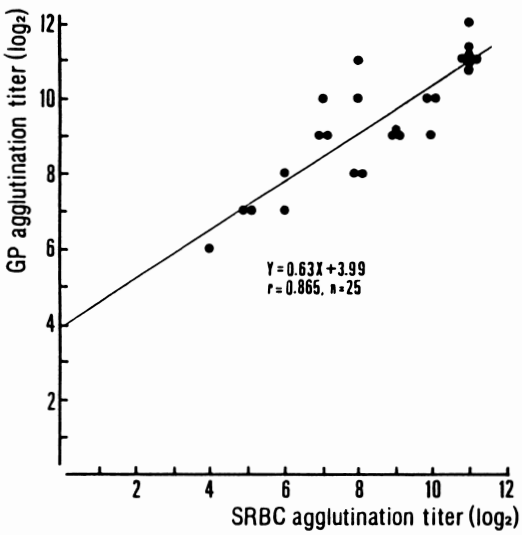

Fig. 2 Correlation between the indirect agglutination titers with gelatin particles and SRBC coated with *S. stercoralis* antigens in 25 patients with *Strongyloides* infection.

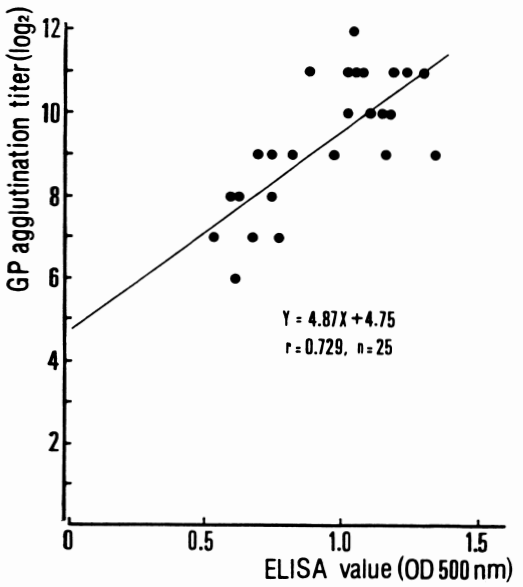

Fig. 3 Correlation between gelatin particle agglutination titers and ELISA values to *S. stercoralis* antigen in 25 patients with *Strongyloides* infection.

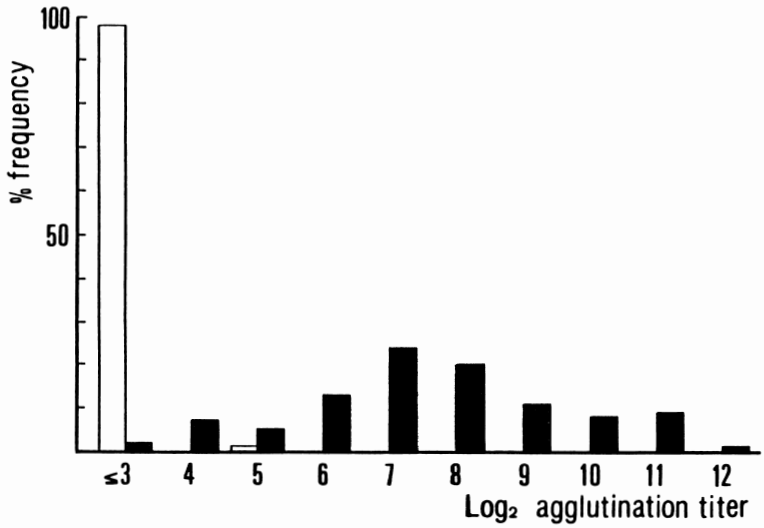

Fig. 4 Percent frequency distributions of agglutination titers estimated with the antigen-gelatin particles in 92 patients with strongyloidiasis (■) and in 60 uninfected controls (□).

a significant correlation ($P < 0.01$) was observed between the results of the two tests. In Fig. 3, the antibody levels assessed by the GPAT and the ELISA were similarly compared and the results also showed a significant relation ($P < 0.01$) between the antibody levels.

The GPAT titers were further estimated on 92 patients and 60 uninfected controls. The results are shown in Fig. 4. More than 97% of the patients gave positive agglutination responses at serum dilutions of over 1:16, whereas in the control subjects, the agglutination titer of 1:32 was detected in only one, and in the remaining 59 persons negative results were demonstrated at the lowest serum dilution of 1:8. In general, the samples which showed positive agglutination at a serum dilution of 1:16 or more are regarded as a positive reaction. Presuming that the criteria is applicable to the present results, false-negative results were observed in only 2.2% (2/92) of the patients with a known infection and a false-positive result was observed in 1.7% (1/60) of the uninfected controls.

Finally, the specificity of the test was determined with the sera from various helminthiases other than strongyloidiasis. As seen in Table 1, a strong positive reaction was observed in one patient with hookworm infection but not in another one. Some patients with anisakiasis, angiostrongyliasis, sparganosis and cysticercosis also gave positive cross-reactions but their reactivities were generally weak, showing titers of 1:32 or less.

Discussion

The diagnosis of strongyloidiasis is definitively made when *Strongyloides* larvae are found in feces and/or in other specimens. In the chronic infections, however, worms are frequently not found in such specimens. In an earlier study, Jones (1950) examined 952 fecal samples from 100 patients with known infection and found the worms in only 27% of the fecal samples. Recently, Grove (1980) has also shown that the parasites were detected by fecal culture in 55% of 44 persons with chronic strongyloidiasis in a first attempt and in only 68% even after two or more attempts. In similar surveys in Okinawa, in which fecal examinations were repeated for three consecutive days, the authors found that the infected persons who were proven positive in all

Table 1 Cross-reactivities of the antigen-coated gelatin particles with sera from various parasitic diseases

Parasitic disease	GPAT titer (Log_2)	Parasitic disease	GPAT titer (Log_2)
Anisakiasis	< 3	Cysticercosis	3
	4		5
	< 3		5
	3		3
	< 3	Unilocular echinococcosis	< 3
Hookworm disease	11	Clonorchiasis sinensis	3
	< 3		< 3
Angiostrongyliasis	4		< 3
	3		< 3
	< 3		< 3
Rhabditis infection	< 3	Schistosomiasis mansoni	< 3
Sparganosis mansoni	5		

GPAT titer: reciprocal agglutination titers determined by the use of the gelatin particles sensitized with *S. stercoralis* antigens.

the examinations were only 27% of the total positive persons, whereas the persons found to be positive in only one out of the three examinations were about 40% (Sato *et al.*, 1984). Therefore, a sensitive and rapid immunodiagnostic procedures to complement the parasitological examinations should be developed for the correct diagnosis of chronic, low-level infection. A number of attempts has been made to develop an immunological test for diagnosis of strongyloidiasis; these have included a skin test (Tribouley-Duret *et al.*, 1976; Sato *et al.*, 1986), an indirect immunofluorescent antibody test (Dafalla, 1972; Grove & Blair, 1981; Genta & Weil, 1982) and an enzyme-linked immunosorbent assay (Tribouley-Duret *et al.*, 1978; Carroll *et al.*, 1981; Neva *et al.*, 1981). The authors have also successfully developed micro-ELISA for strongyloidiasis and have in practice tried to use the assay as a screening test for mass-examination for strongyloidiasis in Okinawa (Sato, 1986). About twelve percent of inhabitants tested were considered to be antibody positive and, when they complied with further fecal examinations, more than half (51.3%) of them were proven to be actually infected with the parasite. Although the ELISA is sensitive enough for detecting *Strongyloides* infection, it involves several experimental steps which are difficult to do in an ordinary laboratory or in a field survey.

In the present study, the authors developed a simple agglutination procedure for serodiagnosis of human strongyloidiasis. The results obtained were quite sensitive and specific for the diagnosis, showing a close correlation with those of the IHAT and the ELISA. The procedure is much simpler than that of the ELISA, because it can be performed by the one-step reaction of the antigen-particles and the test serum. Furthermore, the test requires no specialized equipment and the processing time is much shorter than in other tests.

The gelatin particles are artificial spherical particles made of gelatin and gum arabic. The particles are actively being employed as an antigen carrier for various diagnostic kits. Because of their inert antigenicity, the test can be performed without previous inactivation and absorption of test serum, as it is in the case of the SRBC carrier. Moreover, the antigen-particles have advantages over latex beads and intact SRBC, since they were colored for convenience for reading of the settling pattern and also since they can be stored lyophilized for a long period.

Thus, the test should be more suitable for mass screening for *Strongyloides* infection than the IHAT and the ELISA.

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