[Jap. J. Parasit., Vol. 32, No. 1, 63-70, Feb., 1983]

The Employment of Radioimmunoassay for the Detection of *Ascaris suum* Antibody in Sera from Patients with Ascariasis

NORIAKI TOHGI*, KO TANAKA*, HIROSHI KAWAMURA*
YUKITAKA MIYACHI† AND MORIYASU TSUJI*

(Received for publication; July 7, 1982)

Key words: *Ascaris suum*, ascariasis, radioimmunoassay, polyethylene glycol

Introduction

We developed a new immunological method, radioimmunoassay (RIA) for *Ascaris suum* protein (Asp) extracted from the body fluid of *Ascaris suum* and measured serum Asp levels in patients with helminthiasis and gastrointestinal diseases (Tanaka *et al.*, 1983). Furthermore, the cross reactivities of Asp antibody with other helminth antigens were measured by RIA. Pelley *et al.* (1976; Hamburger *et al.* 1976) extracted major serological antigens from *Schistosoma mansoni* eggs and developed a RIA for these antigens. They also presented a new serodiagnosis method with RIA to detect the antibody titer for one of these antigens in sera from patients with schistosomiasis mansoni (Pelley *et al.*, 1977). In this report, we explored the possibility if a RIA using ^{125}I labelleled Asp could be sensitive and specific method to detect the antibody titer in sera from patients with ascariasis.

Materials and Methods

1) *Preparation of* Ascaris suum *protein (Asp)*, Toxocara canis (*T. canis*), *anti-Asp sera, and anti-*T. canis.

The body fluid of adult *Ascaris suum* worm was filtered through Whatman No. 1 paper (Whatman Co. Springfield), centrifuged at 3,000 rpm for 10 min and lyophilized. The lyophilized antigen was further applied to Sephadex G-200 (2.5×40 cm, Pharmacia Co. Uppsala) and eluted with 0.05 M saline at 4 C. The second protein peak was lyophilized after dialysis and used as the partially purified antigen: *Ascaris suum* protein (Asp) (Tanaka *et al.*, 1983). *T. canis* antigen was prepared from 0.05 M saline extracts of worms according to the method published by Tsuji and Yokogawa (1974); Tsuji (1975).

Anti-Asp serum and anti-*T. canis* serum were prepared by the following method. Emulsions containing 2 mg of Asp or *T. canis* antigen in Freund's complete adjuvant (Difico Co. Detroit) were injected into proximal limbs of rabbit intramuscularly every week ten times. Anti-Asp and anti-*T. canis* sera were obtained from these rabbits one week after the final injection (Tsuji and Yokogawa, 1974; Tsuji, 1975).

2) *Sera from patients*

10 normal control sera; taken from healthy male subjects aged from 24 to 38 years. Sera from 22 cases of patients with

* *Department of Parasitology, Hiroshima University School of Medicine, Hiroshima 734 Japan.*
† *Shizuoka General Hospital, Shizuoka 420 Japan.*

ascariasis; the diagnosis was made by the demonstration of eggs and/or worms. Sera from 3 cases of patients with toxocariasis; the diagnosis was made by the clinical findings, immunoserological tests and the demonstration of eggs in the house dust and/or in the dung of house dogs and cats. Sera from 6 cases of patients with paragonimiasis westermani; the diagnosis was made by the clinical findings and immunological tests, in some cases by the morphological demonstration. Sera from 3 cases of patients with taeniasis saginata; the diagnosis was made by the demonstration of worms or eggs. All sera were stored at −20 C until used.

3) *Detection of serum Asp antibody by RIA*

A lactoperoxidase method (Miyachi *et al.*, 1972, 1977; Sato *et al.*, 1976) was used to prepare ^{125}I-Asp and give the specific activity ranged at 50–100 μCi/μg. The various diluted solutions of anti-Asp rabbit serum, anti-*T. canis* rabbit serum or human serum and approximately 30,000 cpm of ^{125}I-Asp were added to assay tubes (7.5× 90 mm). A total incubation volume was adjusted to 1 ml with 1/15 M phosphate buffer saline, pH 7.4 (Sanko pure chemical Co. Tokyo) containing 0.1% normal rabbit serum. After incubation at 4 C for 16–24 hours, 1 ml of 20% polyethylene glycol T6,000 (Katayama chemical Co. Osaka) and 50 μl of normal human serum were added to each tube. The tubes were mixed and after 10 min centrifuged at 2,500 rpm for 15 min. The supernatants were aspirated and the radioactivities in precipitates were counted by a well type gamma-spectrometer (Fig. 1).

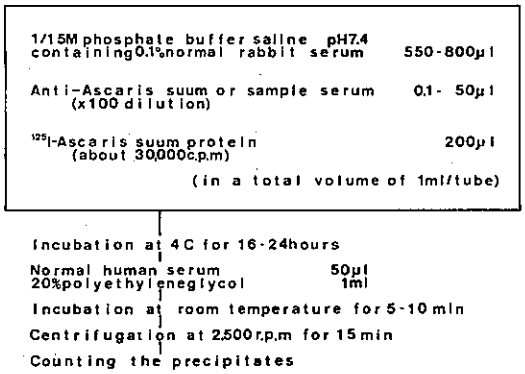

Fig. 1 A procedure of radioimmunoassay for detecting serum *Ascaris suum* protein antibody.

4) *Detection of serum Asp by RIA*

The determination of Asp in sera from human subjects by RIA was performed as previously described (Tanaka *et al.*, 1978). Briefly, 0.5–500 ng of Asp or human serum samples, ^{125}I-Asp and anti-Asp rabbit serum (a final tube dilution 1:25,000) were added to assay tubes and mixed. After incubation at 4 C for 16–24 hours, the sheep anti-rabbit serum (the second antibody) was added to each tube and mixed. After the additional incubation at 4 C for 24 hours, the tubes were centrifuged at 2,500 rpm for 15 min and the radioactivities in precipitates were counted. Sample values were extrapolated from a standard curve.

We also measured serum Asp levels using polyethylene glycol for the precipitation of the antibody bound ^{125}I-Asp instead of the second antibody. After the first incubation, 1 ml of 20% polyethylene glycol and 50 μl of human control serum were added to each tube and vortexed. Afterthen the tubes were centrifuged at 2,500 rpm for 15 min and the radioactivity of precipitates in each tube was counted.

Results

The binding curves of ^{125}I-Asp to anti-Asp rabbit serum, anti-*T. canis* rabbit serum and sera from human subjects are shown in Fig. 2. The vertical and horizontal lines in Fig. 2 present the percent binding of antibody bound ^{125}I-Asp against the total ^{125}I-Asp and the serum volume

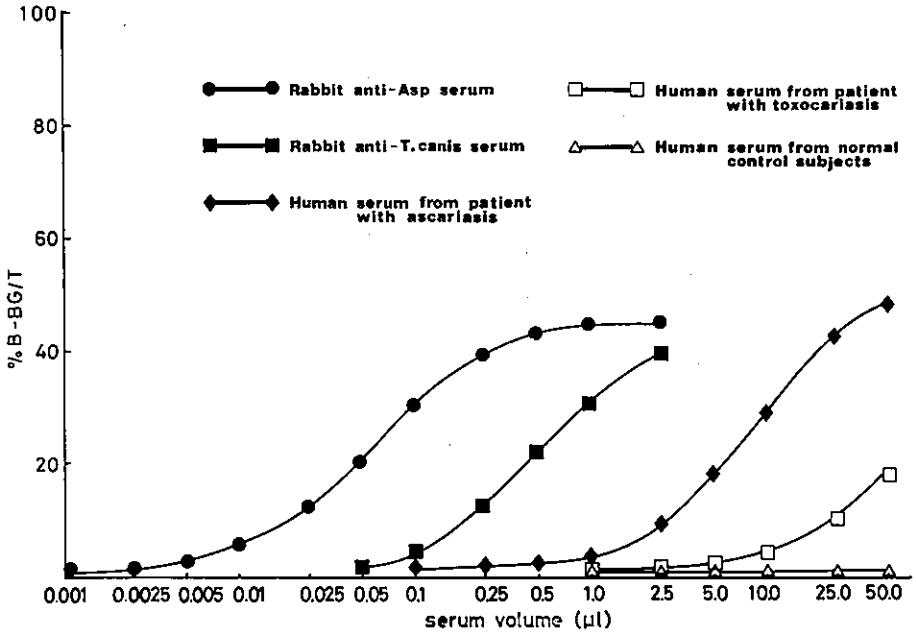

Fig. 2 The binding curves of ^{125}I-*Ascaris suum* protein to rabbit or human serum.

in log dose, respectively. The binding curve of anti-Asp rabbit serum was sigmoid, and the maximum binding of ^{125}I-Asp was obtained in the presence of 2.5 μl of the serum. The serum volume to cause a 50% binding of maximal binding of ^{125}I-Asp, which was designated the B-50, was 0.06 μl. The binding curve of serum from patient with ascariasis was also sigmoid, and the serum volume in B-50 was 8 μl. The slopes of the binding curve of anti-*T. canis* rabbit serum and serum from patient with toxocariasis were sigmoid, and the serum volumes in B-50 were 0.4 and 15 μl, respectively. The dilution curves of normal control sera were identical to the horizontal line in Fig. 2, and no binding of ^{125}I-Asp was observed even when using 50 μl of serum.

In order to represent the antibody titer for Asp more clearly, we converted the serum volume of B-50 to the reciprocal according to the following formula:

$$\text{Asp antibody titer} = \frac{1}{\text{the serum volume of B-50 } (\mu l)} \times 100$$

Fig. 3 shows the Asp antibody titers in sera from normal control subjects and patients with helminthiasis. None of Asp antibody was detected in sera from all cases of control subjects (n=10). High Asp antibody titers were observed in 4 out of 22 cases of ascariasis. In toxocariasis, one of three cases provided a high titer of Asp antibody. None of Asp antibody was observed in sera from 8 cases of anisakiasis, 6 cases of paragonimiasis westermani or 3 cases of taeniasis saginata.

Fig. 4 shows the serum concentration of Asp from normal control subjects and patients with helminthiasis by RIA using the second antibody method. All of the serum levels of Asp from control subjects were below the sensitivity of the RIA (10 ng/ml). High concentrations of Asp

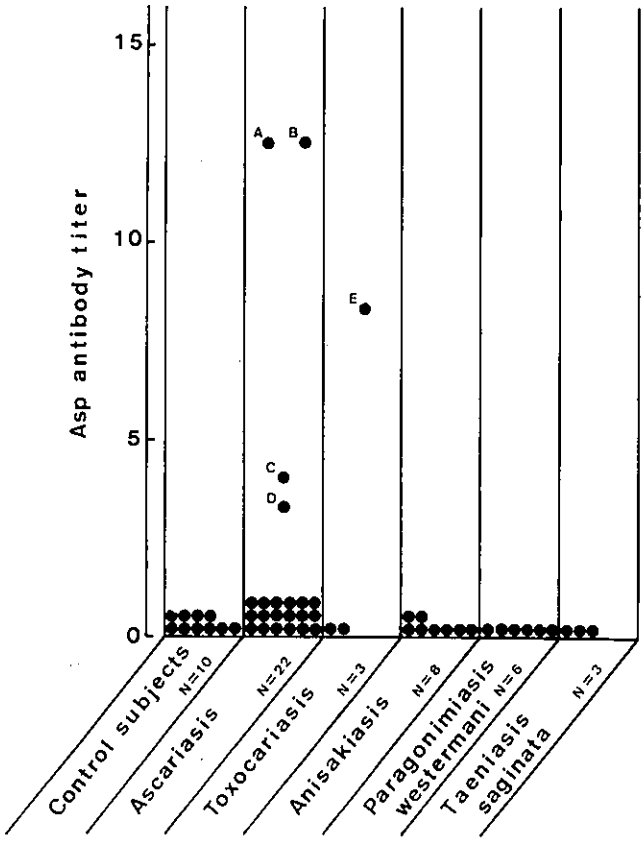

Fig. 3 *Ascaris suum* protein antibody titers in sera of human normal control subjects and in sera of patients with ascariasis and other helminthiasis.

were observed in sera from 6 cases of ascariasis. It is of interest that case A, B, C, D which had very high serum Asp levels, also represented high titers of Asp antibody in sera. Two of three cases of toxocariasis provided high serum concentrations of Asp, one of which also showed high Asp antibody titer (case E). High concentrations of Asp were detected in sera from all cases (n=8) of anisakiasis, two cases of paragonimiasis westermani (n=6) and one case of taeniasis saginata (n=3).

We compared the serum concentration of Asp using the second antibody method and those using polyethylene glycol method. Unfortunately, by RIA using polyethylene glycol, we could not measure all of the samples with high Asp antibody titer because of the lack of serum volume (case A, B, and D). Serum Asp concentrations in case C and E were not detectable by RIA using polyethylene glycol.

Discussion

We have developed the method to detect Asp antibody using RIA and quantitated the Asp antibody titer by conversion of the serum volume of B-50 to the reciprocal. Asp was a partially purified antigen from the body fluid of *Ascaris suum*. A preliminary study with polyacrylamide gel electrophoresis and Sephadex gel chromatography demonstrated that almost all of

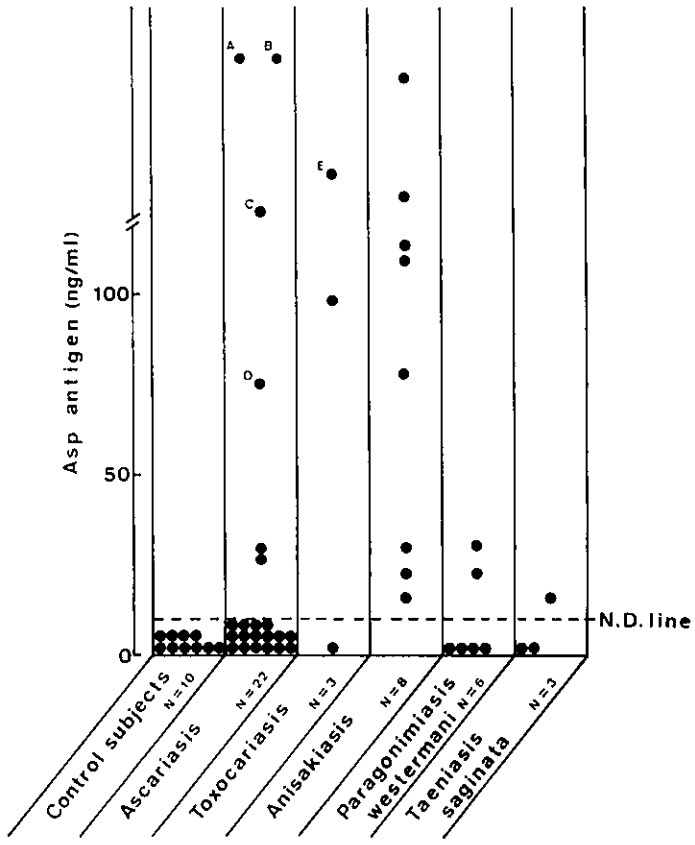

Fig. 4 *Ascaris suum* protein levels in sera of human normal control subjects and in sera of patients with ascariasis and other helminthiasis.
The N.D. line represents the non-detectable amount (10ng/ml) in the RIA.

iodinated Asp was recovered in one peak fraction corresponding to the molecular weight of 15,000 and possessed a high immunoactivity to Asp rabbit antibody (Tanaka *et al.*, 1978). The investigation with RIA has shown that Asp antigenic substance was present in the body fluid of *Ascaris lumbricoides* at the same concentration as *Ascaris suum* (Tanaka *et al.*, 1983). Therefore, Asp antibody present in sera from patients with ascariasis detected by this method may reflect the present or past exposure of patients to Asp antigenic substance of *Ascaris lumbricoides*.

High titers of Asp antibody were observed in only 4 out of 22 cases of ascariasis in our study. O'Donnell and Mitchell (1980) also presented a similar report that of 5 cases from an *Ascaris lumbricoides* endemic area who were positive for *Ascaris* infection (past or current), only 1 contained detectable levels of *Ascaris* specific serum IgG antibodies. These findings suggest that immunodiagnosis based on *Ascaris* antibody in human serum would be useless for a screening method for ascariasis. On the other hand, Pelley *et al.* (1977) reported a very high incidence of seropositive data for schistosomiasis by RIA using *Schistosoma mansoni* egg antigen.

This finding is probably due to the more easy exposure of patients to *Schistosoma mansoni* egg antigen, compared to those with ascariasis to adult worm antigens containing Asp.

It is of interest that patients with ascariasis (case A, B, C, D) containing a high Asp antibody titer also represent a very high serum Asp concentration (over 50 ng/ml) by RIA using the second antibody method. But Asp concentrations were undetectable in these sera when the polyethylene glycol was used for the precipitation of ^{125}I-Asp antibody complex in RIA. This contradiction is probably explained by the following reason. In RIA for detecting Asp antigen, ^{125}I-Asp, anti-Asp rabbit serum and human serum samples are contained in RIA tube. When a high concentration of Asp antibody is present in human serum, this human antibody will compete with the ^{125}I-Asp binding to anti-Asp rabbit antibody, and the reduced amount of ^{125}I-Asp-rabbit antibody complex was recovered in the precipitate by the second antibody method (sheep anti-rabbit IgG serum). On the other hand, ^{125}I-Asp-rabbit antibody complex and ^{125}I-Asp-human antibody complex were precipitated by RIA using polyethylene glycol. These findings suggest that high serum Asp concentrations measured by RIA using the second antibody is probably induced by the presece of a high titer of Asp antibody in human serum. In other 2 cases of ascariasis containing high Asp concentrations (17.5 and 19.5 ng/ml), Asp antibody was not detected even when using 50 μl of human serum.

Among three patients with toxocariasis, one case presented positive data for both Asp antigen and Asp antibody. As well as ascariasis, serum Asp antigen was undetectable when measured by RIA using polyethylene glycol in this case. In the other case, only high Asp concentration was observed. *Toxocara canis* had a high concentration of Asp antigenic substance partially cross reacting with Asp antibody (Tanaka *et al.*, 1983). In toxocariasis known as visceral larvae migrans (Beaver *et al.*, 1952), antigenic substances were easily released to blood stream from the body of *Toxocara* larvae. Therefore, Asp like antigenic substances or the antibody reacting with ^{125}I-Asp may be frequently detected in sera from patients with toxocariasis.

None of Asp antibody was observed in sera from human normal subjects or patients with anisakiasis, paragonimiasis westermani or taeniasis saginata, while high concentrations of Asp were detected in sera from some of these patients. It was suggested from these findings that RIA for detecting Asp antibody appears to be a specific method for patients with ascariasis and toxocariasis.

In conclusion, RIA for detecting serum Asp antibody may be of limited practical applicability because of the false negative results, but the specificity for ascariasis and toxocariasis is acceptable. In addition, it was also indicated that Asp antibody in human serum easily causes a false positive data in RIA for detecting Asp antigen using the second antibody method.

Summary

We have developed the method using radioimmunoassay (RIA) to detect the antibody in human serum against *Ascaris suum* protein (Asp) extracted from the body fluid of *Ascaris suum*. This method was performed by incubation of ^{125}I labelleled Asp with serum samples and precipitation of antibody bound ^{125}I-Asp by polyethylene glycol. In this study, high titers of Asp antibody were observed in 4 out of 22 cases of ascariasis. Among three patients with toxocariasis, one case presented positive data for Asp antibody. None of Asp antibody was observed in

sera from human normal subjects (n=10) or patients with anisakiasis (n=8), paragonimiasis westermani (n=6) or taeniasis saginata (n=3).

We previously reported that significant high levels of Asp antigen were observed in sera from patients with ascariasis and toxocariasis by RIA. In this study, we found that patients with a high titer of Asp antibody in serum also presented a high serum Asp concentration by RIA using the second antibody method, this binding was probably caused by the reason that the Asp antibody in human serum will compete with the ^{125}I-Asp binding to anti-Asp rabbit antibody, and that the amount of ^{125}I-Asp-rabbit antibody complex is reduced.

RIA for detecting serum Asp antibody may be of limited practical applicability because of the false negative results, but the specificity for ascariasis and toxocariasis is acceptable.

References

1) Beaver, P. C., Synder, C. H., Carrera, G. H., Dent, J. H. and Lafferty, J. W. (1952): Chronic eosinophilia due to visceral larva migrans. Pediatrics, 9, 7–19.
2) Hamburger, J., Pelley, R. P. and Warren, K. S. (1976): *Schistosoma mansoni* soluble egg antigens: Determination of the stage and species specificity of their serologic reactivity by radioimmunoassay. J. Immunol., 117, 1561–1566.
3) Miyachi, Y., Vaitukaitis, J. L., Nieschlag, E. and Lipset, M. B. (1972): Enzymatic radioiodination of gonadotropins. J. Clin. Endocrinol., 34, 23–28.
4) Miyachi, Y., Mizuchi, A. and Sato, K. (1977): Preparation of iodinated cyclic GMP derivatives by a lactoperoxidase method. Anal. Biochem., 77, 429–435.
5) O'Donnell, I. J. and Mitchell, G. F. (1980): An investigation of the antigens of *Ascaris lumbricoides* using a radioimmunoassay and sera of naturally infected humans. Int. Arch Allergy Appl. Immunol., 61, 213–219.
6) Pelley, R. P., Pelley, R. J., Hamburger, J., Peters, P. A. and Warren, K. S. (1976): *Schistosoma mansoni* soluble egg antigens: I Identification and purification of their major antigens, and the employment of radioimmunoassay for their further characterization. J. Immunol., 117, 1553–1569.
7) Pelley, R. P., Warren, K. S. and Jordan, P. (1977): Purified antigen radioimmunoassay in serological diagnosis of *Schistosoma mansoni*. Lancet, 781–785.
8) Sato, K., Miyachi, Y., Mizuchi, A., Ohsawa, N. and Kosaka, K. (1976): Enzymatic radioiodination of succinyl cyclic AMP tyrosine methyl ester by lactoperoxidase and radioimmunoassay for cyclic AMP. Endocrinol. Jpn., 23, 251–257.
9) Tanaka, K., Miyachi, Y., Tsuji, M. and Miyoshi, A. (1978): Radioimmunoassay for *Ascaris* specific protein and its clinical approach to gastrointestinal diseases. Jpn. J. Gastroenterol., 75(11), 1832–1839.
10) Tanaka, K., Kawamura, H., Tohgi, N., Tsuji, M., Miyachi, Y. and Miyoshi, A. (1983): The measurement of *Ascaris suum* protein in sera from patients with helminthiasis and with gastrointestinal diseases by radioimmunoassay. Parasitology (in the press).
11) Tsuji, M. and Yokogawa, M. (1974): Immunological diagnosis of helminthic infections. Proc. SEAMEO/Trop. Med. Tech. Meeting, 180–219.
12) Tsuji, M. (1975): Comparative studies on the antigenic structure of several helminthes by immunoelectrophoresis. Jan. J. Parasit., 24, 227–236.

Radioimmunoassay 法を用いた血清特異，*Ascaris suum* 抗体検出

東儀宣哲　田中　孝　河村　寛　辻　守康
（広島大学医学部寄生虫教室学）

宮地幸隆
（静岡県立総合病院）

私達は radioimmunoassay (RIA) 法を用いて豚蛔虫体腔液より抽出した *Ascaris suum* protein (Asp) に対する抗体を検出する方法を確立した．本法は ^{125}I-Asp と被検血清とを反応させた後，polyethylene glycol によって ^{125}I-Asp に結合した抗体を沈澱させその放射能から Asp 抗体価を算出するものである．人蛔虫症22例中４例にまた犬蛔虫症３例中１例に高抗体価の Asp 抗体価の抗体が認められた．一方，正常人血清（10例），アニサキス症（８例），ウエステルマン肺吸虫症（６例），無鉤条虫症（３例）の患者血清のいづれにも Asp 抗体は認められなかった．

私達は以前に RIA 法を用いて高値の Asp 抗原が人蛔虫症，犬蛔虫症患者血清に認められたことを報告した．今回，私達は人血清中の高抗体価の Asp 抗体が第２抗体法を用いた Asp 抗原を検出する RIA 法において偽陽性反応を引き起こすことを見出した．これは，人血清中に存在する人 Asp 抗体が抗 Asp 家兎抗体と競合して ^{125}I-Asp と結合し，^{126}I-Asp 結合家兎抗体複合体量が減少するためである．

人蛔虫症では本法の陽性例は少なく本法を人蛔虫症の血清学的診断に用いることは困難と考えられる．しかし本法の陽性例は人蛔虫症，犬蛔虫症に限定されその特異性に関してはほぼ満足のゆく結果が得られた．また人あるいは犬蛔虫症の血清 Asp 抗原値を RIA 法で測定する場合，Asp 抗体の存在を留意しなくてはならない事が示唆された．