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Radioallergosorbent Test for Human Filariasis Using Purified *Dirofilaria immitis* Antigen (FST)

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Introduction

Diagnosis of filariasis is generally worked by the detection of microfilariae (mf) in patients' peripheral blood. The method is, however, not only laborious and time consuming, but also not suitable to diagnose patients who are infected with small number of worms. Immunodiagnostic methods for filariasis have been developed by many workers as reviewed by Kagan (1963). Since 1962, Sawada and his associates (1965, 1969, 1975) have reported antigens FST and FST3 which were purified product from *Dirofilaria* (*D.*) *immitis*. The purified antigens were useful to exert immediate type of skin reaction at high positive rate. About 92.3% people showing mf of *Wuchreria* (*W.*) *bancrofti* and with clinical symptoms of filariasis manifested immediate type of reaction after skin injection of the antigens (Sawada *et al.*, 1969). Smith *et al.* (1971) studied the usefulness and limitations of FST antigen in the diagnosis of filariasis. They compared (1) results in a group not exposed to filariasis and effects of repeated testing—England (2) results in a group not exposed to filariasis, but with various intestinal worm infections—USSR and (3) results in groups in areas where *W. bancrofti* infection is endemic —Burma, India, Tanzania. With such background studies, the authors concluded that the antigen could be used to help diagnosis of individual patients as well as to apply to a preliminary screening test in population groups.

Radioallergosorvent test (RAST) for the measurement of specific IgE antibodies reported by Wide *et al.* (1967) has been used widely for diagnosis of allergic diseases caused by many kinds of allergens. In the field of parasitology, Huldt *et al.* (1973) reported that RAST was excellent method for the detection of specific IgE in granular echinococcosis. Ito *et al.* (1977) used RAST in the diagnosis for the multilocular echinococcosis and proposed to conduct several immunologic tests on the same sera to obtain higher accuracy of the results. Because of the simplicity, skin test has been

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appreciated in the practical diagnosis of patients, however, several disadvantages are also pointed out. The latent danger of repeated injection of the antigen, possible contamination of any harmful organisms or substances have not yet been totally excluded. The results are occasionally not objective especially when the reactions are at lower limit. Hence some serological alternative measure is desired so that several comparative immuno-diagnostic tests can be concurrently conducted on the same test sera. Immediate type skin reaction will be replaced by measuring specific IgE with the use of some serological tool.

The present paper deals with the usefulness of purified antigen from *D. immitis* in measuring specific IgE in human filariasis cases in the RAST system. Sera from patients showing microfilariae in the peripheral blood obtained in the endemic area in Malaysia were tested using FST3 and FST3-1. The results are promising in the present study.

It was also shown in the present experiment that IgE from filarial patient reacted with protein fraction which showed globinolytic activity of the worm. The finding was notable from the viewpoint of detecting antigenic substance released from living parasite harbouring in the host.

Materials and Methods

(1) Preparation of antigens FST3, and FST3-1 from *D. immitis*. Adult worms of *D. immitis* were collected from infected dog hearts and lyophilized after washing three times with saline. Antigen FST3 was obtained from lyophilized worms according to the method described by Sawada *et al.* (1969). Antigen FST3-1 was the eluate from polyacrylamide gel column after disc electrophoresis of antigen FST3 (Sato and Sawada ,1969). These antigens were lyophilized and stored at −20 C.

(2) Preparation of globinolytic enzyme from *D. immitis* adult worms. Globinolytic enzyme was prepared following the reported precise method described by Sato *et al.* (1978). Briefly, *D. immitis* adult worms were taken out of the hearts of infected dogs. The worms were homogenized in 0.1 M phosphate buffer, pH 7.2 followed by sonication at 10 KC for 30 minutes. The parasite homogenate was centrifuged at 14,000 rpm for 30 minutes. The resulted supernatant was filtrated through Sephadex G-200. Measurement of enzymatic activity was worked principally according to the method by Sauer and Senft (1972). Enzymatic activity was found in the first peak of protein distribution spectrum. The fractions showing the enzymatic activity were pooled and applied to CM cellulose column chromatography. The enzymatic activity was detected in the fractions eluted by 0.05 M phosphate buffer containing 0.1 M NaCl, pH 7.2. The obtained eluate was further purified through DEAE Sephadex; the enzymatic activity was found in two peaks of protein solution eluted by 0.02 M phosphate buffer containing 0.1 M NaCl, pH 6.5 (Enzyme I) and by the buffer containing 0.25 M NaCl, pH 6.5 (Enzyme II).

(3) Sera. Sera from microfilariae carriers were collected in endemic area of Malaysia. Sera from patients of ascariasis, paragonimiasis miyazakii were collected from infected humans in Juntendo Hospital (Tokyo). Sera from patients of schistosomiasis mansoni were obtained in Brazil. Normal sera were taken from healthy individuals living in a non-endemic area of Japan.

(4) Radiallergosorbent Test (RAST). The paper disc method of RAST was carried out according to the method described by Ceska *et al.* (1972). Antigen solutions of FST3 and FST3-1 were used at the concentration of 100 micro gramme protein/ml according to the method described by Aoki (1980). Protein level was measured according to the method described by Lowry *et al.*

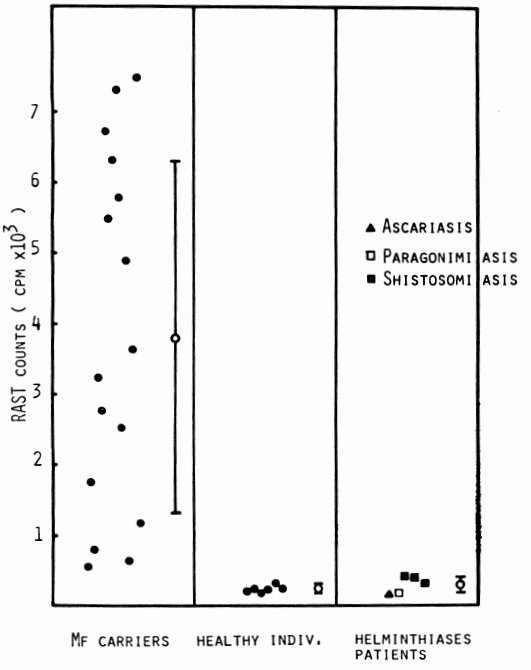

Fig. 1 RAST counts with antigen FST3 against sera from mf carriers and healthy individuals.

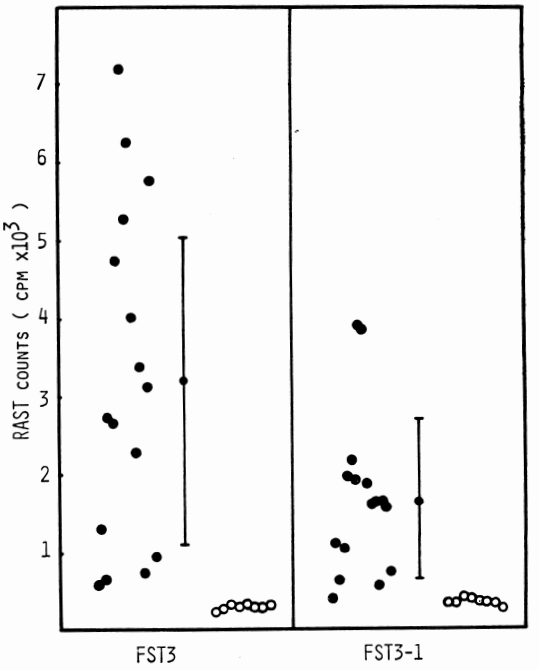

Fig. 2 RAST counts with FST3 and FST3-1 against sera from mf carriers (closed circle) and healthy individuals (open circle).

(1951). Calibulation curve was worked out using bovine serum albumin as the reference protein.

Results

I. Results of RAST using purified skin test antigen FST3

In order to examine the applicability of the filarial skin test antigen FST3 to RAST system, sera from microfilaria carriers in Malaysia were studied by the RAST system. As is shown in Figure 1, RAST counts obtained from 16 sera show wide distribution pattern ranging from 554 to 7,468 cpm (mean: 3,814±2,483 cpm). When RAST was carried out against sera from healthy individuals using the same paper disc, cpm counts were 195–312 cpm (mean: 223±48 cpm) and the distribution of each value was limited. The difference of RAST counts between sera from microfilariae carriers and healthy individuals were obvious as is shown in the Fig. 1. Sera from patients with other helminthic infections showed the similar values to those given by sera from healthy individuals in the same system. From these results, it was suggested that skin test antigen FST3 would be applicable to RAST for the diagnosis of filariasis, although the number of sera examined was limited.

II. Results of RAST with the single band-skin test antigen FST3-1

Filarial skin test antigen FST3-1 is the purified product from FST3 through disc electrophoresis. Not less than 95% of the FST3-1 protein consisted of the antigenic substances of molecular weight 20,000 (Sawada *et al.* 1975). Excellent compatibility has been observed to date between the positive skin reaction elicited by FST3-1 with clinical manifestations of filariasis (unpublished data). The reactivities of FST3-1 and FST3 were comparatively studied by RAST using sera from microfilaria carriers

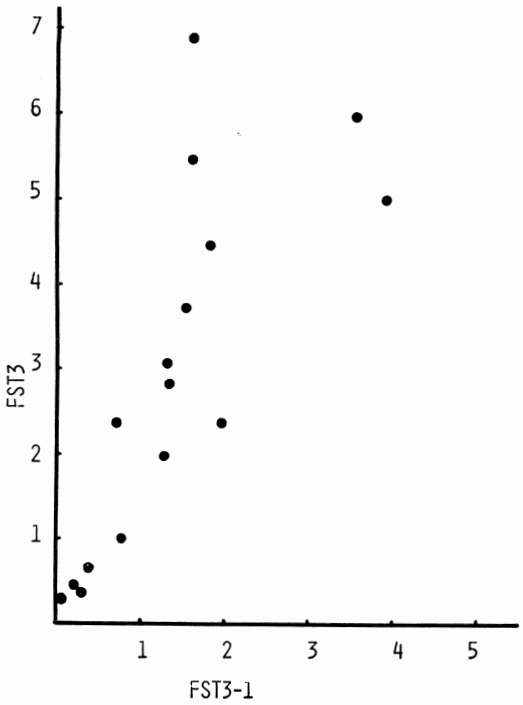

Fig. 3 Correlation between the RAST counts with antigen FST3 and FST3-1 against the same sera from mf carriers.

and sera from healthy individuals (Fig. 2). Higher cpm counts of FST3 were recorded than those shown by FST3-1 against filarial sera, although both antigens showed significantly low reactivity with the sera from healthy individuals. Correlation between the RAST counts with antigen FST3 and FST3-1 against respective sera from microfilaria carriers is demonstrated in Figure 3. Relationship between the reactivity of FST3 and FST3-1 was given in the equation: Y=2X where X is cpm counts by FST3-1 system, Y is cpm counts by FST3 system. Hence the obtained counts using FST3 was 2 times higher than that shown by FST3-1 when the same sera were studied by RAST. The results coincided with our previous findings that wheal size elicited in the patients using FST3 was always larger than those caused by FST3-1 injection.

III. Results of RAST partially purified

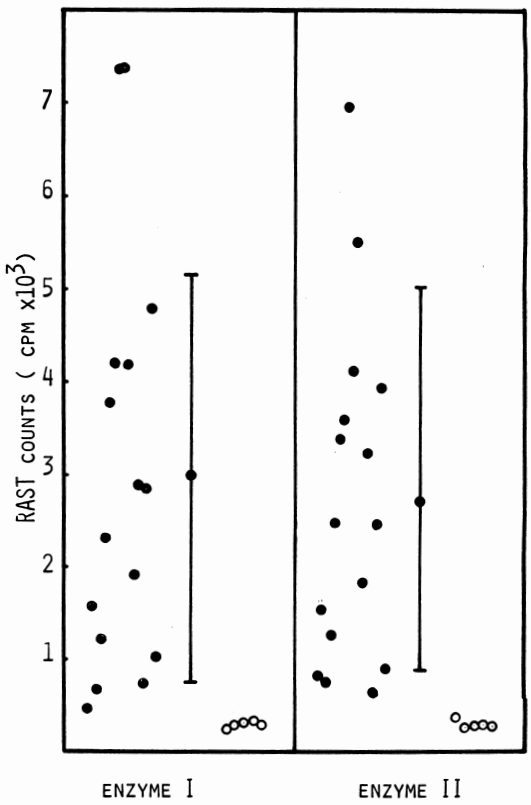

Fig. 4 RAST counts with globinolytic enzymes from *D. immitis* against sera from mf carriers (closed circle) and healthy individuals (open circle).

globinolytic enzyme

Detection of enzyme produced by active parasite would lead an identification of live parasite harbouring in an infected host. When the infection is latent and detection of microfilaria is difficult, immunological method to detect some specific enzyme released by live worms but not from the infected host will give answer for the active infection. Two types of globinolytic enzyme, enzyme fraction I and enzyme fraction II extracted from *D. immitis* were studied in the present experiment for the detection of IgE which would specifically bind the enzymes. The prepared enzyme fraction I and fraction II were adsorbed on paper disc and processed for RAST using the same patient sera. Results are shown

in Figure 4. Distribution profile of the RAST counts shown by the same 16 sera were comparable to the results obtained using FST3, while sera from healthy individuals showed very limited reaction if any.

Discussion

Human filariasis has been diagnosed by direct demonstration of microfilaria in the peripheral blood. Skin test has also been introduced in mass examination among the endemic population. Antigen FST3 which was extracted from *D. immitis* and highly purified by biochemical methods was recommended for this purpose by World Health Organization and cited in Manson's Tropical Diseases (1972).

The usefulness of FST3 in the study on detecting specific IgE has been shown in the present study. Although the skin test is one of the most useful method in mass or individual diagnosis for filariasis, direct demonstration of specific IgE in patients will claim several advantages; first, non-specific reaction associated with intradermal injection will be excluded; the differences of reactivity among the races would be neglegible factor in the RAST. Second, results will be much more objective and differences in the results caused by technical error will be minimized. And third, RAST can be studied on a serum concurrently with other serodiagnosis methods.

So far as examined, the promising specificity and sensitivity of FST3 and FST3-1 in measuring specific IgE level by means of RAST were observed in the present study. The higher cpm level shown in the RAST with the use of FST3 will be explained as follows; FST3-1 is the product of higher purification step from FST3. It is considered that FST3-1 consists of less number of antigenic component than FST3 which may contain a variety of antigenic proteins corresponding to each specific IgE. The high purity caused lowered number of reactive proteins of the antigen both in RAST and in skin test, which led to lowered reactivity of the FST3-1 antigen.

Cross reactivity of antigens extracted in veronal buffer (Chaffee, 1954) prepared from *S. japonicum, S. mansoni, Fasciola hepatica* did not manifest significant reactivity with human filarial sera in the RAST, however, the antigen extracted from *Angiostrongylus cantonensis* provoked a considerable reaction with the human filarial sera. The explanation for this phenomenon has not yet been given. In any serological test, detection of specific antibody against some specific substance released from live parasite will lead the diagnosis of latent active parasitization. Globinolytic enzyme extracted from some worms has been studied by several workers and Senft and Maddison (1975) demonstrated the specific reactivity of globinolytic enzyme when injected intradermally into infected human cases. The present results have also suggested the usefulness of globinolytic enzyme for RAST antigen for the immuno-diagnosis of human filariasis. It was suggested that enzyme fraction from *D. immitis* can be used in place of the enzyme from filarial worms infected in human patients so far as the present experiment concerns. Further purification of the enzyme from *D. immitis* and from human filarial worms will be required before the practical application of the present results.

Summary

Usefulness of purified *Dirofilaria immitis* antigen for assay of specific filarial IgE in 16 human filariasis cases was preliminarily studied by radioallergosorbent test (RAST) under the comparison with 11 sera from healthy individuals and patients with ascariasis, paragonimiasis and with schistosomiasis. Although the studied sera were

limited, acceptable specificity was observed in the RAST system using purified skin test antigen FST3. When FST3-1 antigen which is the product of higher purification step was used, the obtained RAST count was lower than the results in FST3 system. It may be that antigen material composed of various proteins from parasite gave more favourable results than highly purified parasite protein in the practical application so far as the specificity was acceptable. It was also suggested that globinolytic enzyme extracted from *D. immitis* bound specifically with IgE in human filariasis. A product of viable parasites seemed to react as an antigen in the detection of specific IgE produced in the active filarial infection.

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フィラリア症診断用皮内反応抗原を用いた Radioallergosorbent test の研究

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蠕虫感染症においては一般に血清中の特異的 IgE 抗体の上昇が認められ，通常 Radioallergosorbent test (RAST) 法によって測定されている．われわれは今回，*Dirofilaria immitis* から精製した皮内反応用抗原が RAST 法の抗原として，フィラリアの感染者血清に特異反応を示すかどうかを検討した．最初に FST3 を抗原として，16名のフィラリア感染者および11名の対象者（健康者，回虫感染者，肺吸虫およびマンソン住血吸虫感染者）の血清について PAST 法を行ったところ，フィラリア感染者血清の cpm 値は対象者血清に比較して明らかに高く，皮内反応抗原 FST3 は RAST 法の抗原としても十分使用できると判断された．次に，FST3 から disc 電気泳動によってさらに精製した抗原 FST3-1 を用いて RAST 法を行った．FST3-1 はフィラリア感染者血清によく反応したが，同一感染者血清について FST3 によって得られた値と比較すると約$\frac{1}{2}$であった．皮内反応においても，同一患者について FST3-1 により生じる膨疹の大きさは，FST3 により生じる膨疹よりも小さいことが確められており（沢田ら，私信），RAST の結果と合致している．このことは FST3 の構成成分が数種の蛋白質から成るのに比し，FST3-1 がほぼ単一の蛋白質から成ることによるものと推測された．次にわれわれは *D. immitis* に含まれる特異的グロビン分解酵素を抗原として RAST 法を試みた．特異的グロビン分解酵素 EI，EII を抗原として本法を行ったところ，FST3 を抗原とした場合の値にほぼ一致し，グロビン分解酵素も RAST 法の抗原として使用可能であることが示唆された．生存中の虫体が産生している酵素に抗原性が認められたということは，本酵素を抗原とした RAST 法がフィラリア症の診断に大きく貢献できる可能性を示している．しかし，今回用いた特異的グロビン分解酵素は部分精製標品であり，さらに精製した酵素について検討する必要があろう．