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Single Radial Immunodiffusion Method for Determination of the Allergen Concentration in *Dirofilaria immitis*

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Abstract: The method for determination of the exact allergen concentration in *Dirofilaria immitis* was reported. The highly purified allergen obtained from *D. immitis* was repeatedly injected to rats with Freund's complete adjuvant and the monospecific antibody against the allergen was got from the rats hyperimmunized. The present experiment showed that the method of single radial immunodiffusion by the use of the monospecific antibody against the allergen determined the exact allergen concentration. The area πR^2 of a precipitation circle, which developed in anti-allergen serum-containing agar gel layer when allergen diffused into this layer, was proved to be directly proportional to the amount of the allergen used.

Key words: Dirofilaria, Allergen concentration, Monospecific antibody.

The conditions which result in the onset of immediate type hypersensitivity reaction in the host infected with helminths are poorly understood. The development of the allergic state requires not only exposure to the allergen but appropriate conditions in the host during or following exposure (Jarrett and Miller, 1982). In order to see the relationship between the host in reaginic conditions and the parasite infected, it is necessary to study the ability of certain antigens in the parasite to provoke reagin responses as well as to know the localized site and the amount of the allergen inside of the parasite infected.

We have separated allergen from adult *Dirofilaria immitis* (Fujita, 1975) and obtained the highly purified allergen (Fujita, Ikeda and Tsukidate, 1979). The allergen obtained was a single protein, since it showed a single band on gel electrophoresis in sodium dodecyl sulfate (SDS) at pH 8.2, and also only one precipitin arc on immunodif-

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fusion test (Fujita and Tsukidate, 1981). The localization of the purified allergen in the worm was then examined by the fluorescent antibody technique and the Ouchterony's immunodiffusion method. The allergen concentrated mainly in excretory and secretory (ES) products exhausted by adult Dirofilaria worm (Fujita and Tsukidate, 1982). The object of the present study is to develope the method for determination of the allergen concentration, in order to know where and how much the allergen is contained in the Dirofilaria worm.

The allergen was purified from the crude extracts of *D. immitis* worms, as reported earier (Fujita and Tsukidate, 1981). The purified allergen was repeatedly injected to rats with Freund's complete adjuvant at 1 week intervals. Bloods of rats were taken 10 days after the 5th injections. The rats hyperimmunized with the allergen produced only IHA antibody with the relatively lower mean ± S.E. titer of $3^{3.0}\pm0$.

The method of single radial immunodiffusion according to Becker, 1969 with slight modification was used for determination of the allergen concentration. The gel plates were made in the following manner: 3g noble agar was heated to about 60°C in 100ml diethylbarbiturate buffer solution pH 8.6. The mixture was cooled to 48°C and to 10ml of the agar solution, 10ml of the hyperimmune anti-allergen rat serum preheated to 48°C was added. After fully mixing, 1.5ml of the solution was poured into a simple base made with two glass plates, and the depth of the antiserum containing gel layer was made to 1.0mm. The gel layer was left to solidify in the refrigerator, and cylindrical wells of exactly 1.0mm diameter were stencilled into the gel. Using a microliter syringe, each well was filled with 5 μl of the solution of the samples containing the allergen.

Serial dilutions of the allergen solution were made from 1mg per ml to 0.025mg per ml with Veronal buffer at pH 8.6 μ=0.05, and they were dropped in a hole of the anti-allergen serum-containing agar gel layer. The profiles of the diffusion of the purified allergen into gel layer were shown in Fig. 1. The squared zone diameter of each of the precipitation circle developed in the agar gel layer was measured and it was directly proportional to the amount of the allergen used, as shown in Fig. 2. The

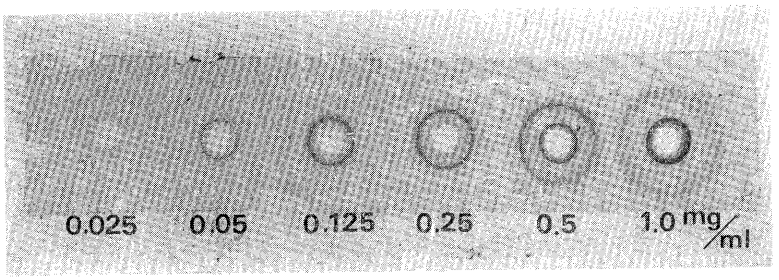

Fig. 1. Diffusion profiles of the purified allergen in anti-allergen serum-containing gel layer. Figures showed the allergen concentration per ml.

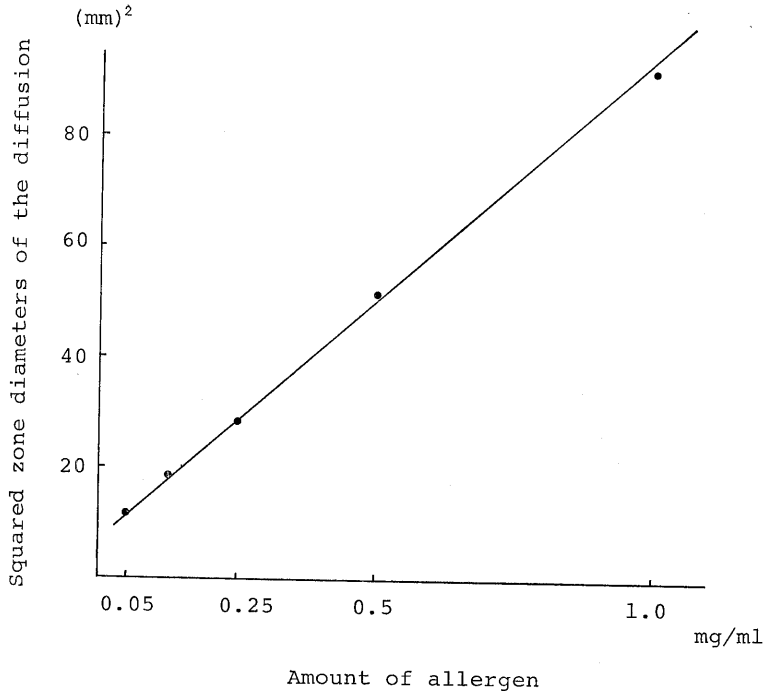

Fig. 2. Single radial immunodiffusion of the purified allergen against the monospecific anti-allergen rat serum and standard curve.

experiment showed that the exact allergen concentration was determined by the single radial immunodiffusion method with the use of the monospecific antibody against the highly purified allergen.

The method reported here was thought to be the simple and useful method for the detection and the determination of the allergen in *D. immitis* worm. It seemed very important to know where and how much allergen was contained in each part of Dirofilaria worm in understanding the host-parasite relationship in the filarial infection.

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犬フィラリア虫体内含有アレルゲン量測定のための Single Radial Immunodiffusion 法

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犬フィラリア虫体のどこに，どれだけのアレルゲンが含まれているかを知ることは，フィラリア症における寄生体・宿主相互関係を理解する上で重要である．われわれは，虫体内のアレルゲン量を正確に測定できる Single Radial Immunodiffusion 法を開発した．

犬フィラリア由来の精製アレルゲンを，Freund の完全アジュバントと共に，ラットに何度も免疫し，精製アレルゲンに対する Monospecific な抗体を作製した．この抗体を含む寒天ゲルをガラス板上に重層し，円柱状の穴をあけて，測定すべき材料を注入した．寒天ゲル内で形成された円形沈降帯の面積，πR^2 はアレルゲン量と正確に比例し，この方法が，フィラリア虫体内に含有するアレルゲン量を正確に測定する方法であることを証明した．

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