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Research Note

Localization of a Thiol Protease in Metacercarial Lung Fluke

FUSANORI HAMAJIMA[1], KAZUO YAMAKAMI[1]
AND TAKAHIRO FUJINO[2]

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Juvenile lung flukes injure various tissues and organs by penetrating and feeding during the migration to the host's lungs (Yokogawa *et al.*, 1960). It was found recently that a thiol protease in the metacercariae of *Paragonimus westermani* hydrolyzes collagen, a major structural protein of mammalian hosts, and hemoglobin (Hamajima *et al.*, 1984; Yamakami and Hamajima, 1985). Therefore, it was thought worthwhile to elucidate the localization of the protease in the metacercariae in connection with the host-parasite relationship (Hamajima *et al.*, 1984; Hamajima and Yamakami, 1985). The present study was attempted to demonstrate the distribution of this protease using an indirect fluorescent antibody technique (IFAT).

Metacercariae of *P. westermani* (triploid type) used in these experiments were isolated from the crabs, *Eriocheir japonicus* collected on the Tsushima Is., Japan. The thiol protease of the larvae was purified by affinity chromatography on arginine-Sepharose CL-4B, gel filtration on Ultrogel AcA-54 and DE-32 column chromatography essentially according to the methods of Yamakami and Hamajima (1985).

The purified enzyme was emulsified with an equal amount of Freund's Complete Adjuvant and injected into the auricular vein of rabbits ten times over a three week period. One week after the last injection, serum was harvested, and it was verified that anti-protease immunoglobulin in the serum was strongly positive by an Ouchterlony double diffusion method. IgG in the serum was prepared by protein A-Sepharose CL-4B affinity chromatography.

For IFAT, procedure of paraffine embedding and IFAT was carried out according to the methods of Sainte-Marie(1962)and Bennett *et al.* (1982) with some modification. IgG applied to sections was diluted 1 : 100 with PBS. FITC-labelled goat anti-rabbit IgG (FITC-GARI) was diluted 1 : 20 with PBS. Sectioned metacercariae were examined under a Olympus fluorescence microscope with epi-ultraviolet illumination.

The IFA method gave a positive fluorescence in the gastrodermis of the larvae (Fig. 1); the fluorescence was seen over the epithelium, brush border and luminal substances in the caecum. The tegument and excretory bladder did not display fluorescence. Control sections treated with normal rabbit IgG at dilutions greater than 1/100, followed by the treatment with FITC-GARI at dilutions greater than 1/20, showed no fluorescence. Similar result was reported for the acid protease in adult *Schistosoma mansoni* and *S. japonicum* (Bogitsh and Dresden, 1983). This observation suggests that the thiol protease secreted in the digestive tract may appear to be of importance for the hemoglobin digestion on feeding in the final host (Hamajima *et al.*, 1984; Yamakami and Hamajima, 1985), and that the enzyme excreated from the tract may possibly be responsible for the degradation of collagen during their migration to the host lungs (Hamajima and Yamakami, 1985;

[1]*Department of Parasitology, National Defense Medical College, Tokorozawa 359, Japan*; [2]*Department of Parasitology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan.*

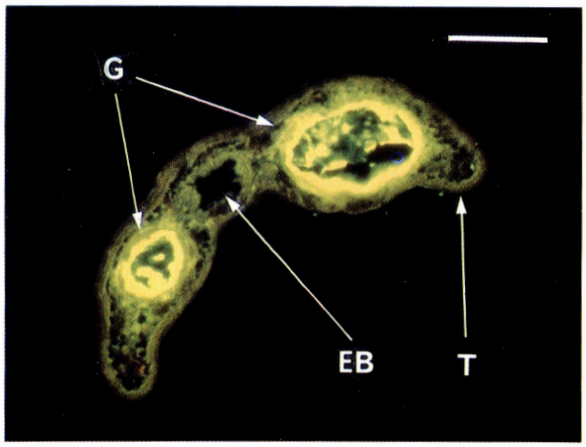

Fig. 1 Cross section of excysted metacercarial larva treated with anti-protease IgG, and reacted with FITC-GARI shows strong fluorescence in the gastrodermis (G), but no fluorescence in the tegument (T) and the excretory bladder (EB). (Scale: 50 μm)

Yamakami and Hamajima, 1985).

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短　報

肺吸虫メタセルカリアにおける蛋白水解酵素の局在

浜島房則[1)]　山上和夫[1)]　藤野隆博[2)]

([1)]防衛医科大学校寄生虫学教室　[2)]九州大学医学部寄生虫学教室)

メタセルカリアの蛋白水解酵素は終宿主への侵入，そこでの移行およびその組織の消化，さらには宿主反応にとって重要な役割を果しているものと考えられる．そこで，本酵素を精製し，これに対する IgG をつくり，IFAT 法で幼虫体における本酵素の分布を検討した．その螢光反応の結果，腸管，特に腸上皮および腸管腔内に本酵素が存在していることが明らかとなった．