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Research Note

Eosinophil Accumulation in Guinea Pigs by a Collagenolytic Fragment Induced by a Metacercarial Protease

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It has been well established that a thiol protease is excreted from the caecum of *Paragonimus westermani* metacercariae and appears to be of importance for migration of the worms to the lungs and provoking host response to the parasite as well (Hamajima and Yamakami, 1985; Hamajima *et al.*, 1985; Hamajima *et al.*, 1986). Although the protease is capable of hydrolyzing collagen, a major connective tissue protein of mammalian hosts *in vitro* (Yamakami and Hamajima, 1985; Yamakami and Hamajima, 1986), the intraperitoneal injection of the protease eventually causes the accumulation of eosinophils in the peritoneal cavity (Hamajima and Yamakami, 1985; Hamajima *et al.*, 1986). Therefore, it would be of interest to determine whether peptide derived from homologous collagen hydrolyzed by the thiol protease could cause the eosinophil accumulation *in vivo*. Thus, the present experiment deals with the eosinophil accumulation in the guinea pig peritoneal cavity following the intraperitoneal injection of the peptide derived from the collagen.

Metacercariae of *P. westermani* (triploid type) were collected from *Eriocheir japonicus*. 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). Insoluble collagen used in this experiment was purified from guinea pig lungs by a modification of a procedure of Deyl and Adam (1976). The collagen-derived peptides were prepared according to the method of Yamakami and Hamajima (1986). The peptide fragments were then separated by reversed phase high-performance liquid chromatography (HPLC) on a column of SynCropak Rp-8 (4.1 × 250 mm) eluted at a flow rate of 1.0 ml/min with 0.1%-trifluoroacetic acid (TFA) for 10 min, and then with a linear gradient of acetonitrile in 0.1%-TFA (Yamakami and Hamajima, 1986).

Normal Hartley strain female guinea pigs weighing 400–500 g and corresponding guinea pigs infected orally with 100 metacercariae were used for the assay of *in vivo* eosinophil accumulation. These normal and infected guinea pigs were divided into two groups; those in one group were given 2.0 ml of the peptide (5.0 μg) intraperitoneally, while the guinea pigs in the other group were injected with physiological saline solution as a control.

Peritoneal exudate cells were harvested from the guinea pigs anesthetized with ether using 30 ml of saline solution containing heparin (10

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units/ml). Peripheral blood was obtained by severing the end of a toe. The eosinophil accumulation assay and the calculation of the data obtained have been described elsewhere (Hamajima *et al.*, 1986). Eosinophil counts for peritoneal exudates and peripheral blood were made at 3 and 6 hr after the injection of the peptide fragments.

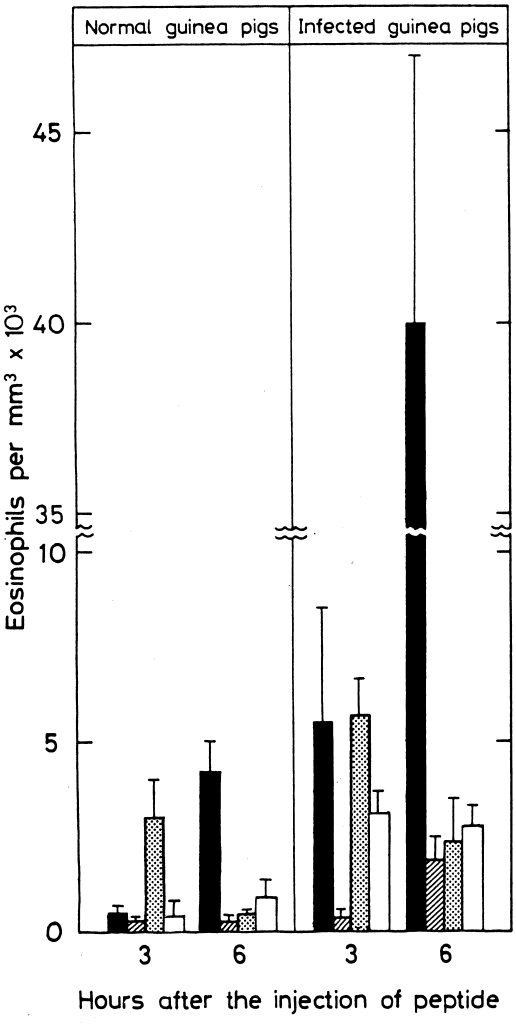

Fig. 1 Eosinophil accumulation in the peritoneal exudates and peripheral blood of normal and infected guinea pigs following the intraperitoneal injection of a peptide fragment derived from collagen hydrolyzed with a thiol protease of *Paragonimus westermani* metacercariae. The results represent the mean ± SE of cell numbers of six animals in each group; the number of peritoneal exudate cells in guinea pigs injected with the fragment (■) or saline solution (▨); the number of peripheral blood cells after the injection of the fragment (▩) or saline solution (□).

A peak peritoneal eosinophilia could be noted when the guinea pigs were injected with a fragment eluted at 47.0 min in retention time by the reversed phase HPLC (Yamakami and Hamajima, 1986).

As shown in Fig. 1, the number of peripheral blood eosinophils increased significantly over the control at 3 hr following the intraperitoneal injection of the fragment in the both normal and infected guinea pigs ($P < 0.05$), while the peritoneal exudate eosinophil counts did not exceed those of the control in either group ($P < 0.2$). Afterwards, the number of peritoneal eosinophils increased markedly over the control at 6 hr following the injection in the both normal and infected animals ($P < 0.01$). On the other hand, the peripheral eosinophil counts did not exceed those of the control in either group ($P < 0.3$–0.7). In addition, Hamajima and Yamakami (1986) reported that chemotactic activity for eosinophils was found in the metacercarial protease and the fragment derived from collagen induced by the enzyme. From these results, the present study suggests that the eosinophil accumulation in the peritoneal exudate of guinea pigs could be mediated by a certain homologous collagen-derived peptide as a chemotactic factor induced by *P. westermani* metacercarial protease, although the mechanism underlying this phenomenon is obscure.

On the other hand, the number of peripheral blood and peritoneal exudate eosinophils in the infected guinea pigs were at higher levels than those from the normal animals ($P < 0.05$–0.01). This result is similar to those reported for the recovery of large numbers of eosinophils from the peritoneal exudates of mice infected with *Toxocara canis* (Sugane and Oshima, 1980) and *P. westermani* (Hamajima *et al.*, 1986). It seems that the high peripheral and peritoneal eosinophil levels in the infected animals may probably be a result of an increase

of bone marrow eosinophil production provoked by helminthic infections as reported previously by Basten *et al.* (1970) and Basten and Beeson (1970).

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短　報

メタセルカリア蛋白水解酵素のコラーゲン水解フラグメントによるモルモットにおける好酸球の集積

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われわれはウェステルマン肺吸虫メタセルカリアの蛋白水解酵素が宿主コラーゲンを水解し，この反応生成物の宿主腹腔内注射が腹腔浸出細胞中に好酸球を遊走集積させることを報告した．しかし，この遊走集積機序について未だ不明な点が多い．そこで，本酵素によってモルモットコラーゲンを基質として *in vitro* で水解し，その反応によって蛋白から遊離した生成物を逆相-高速液体クロマトグラフィー（HPLC）でペプチドフラグメントに分離し，これをモルモット腹腔内に注射し，末梢血中および腹腔内における好酸球数を検討した．その結果，本水解物のHPLCにおけるリテンションタイム47.0 minのフラグメントは末梢血中および腹腔内に好酸球を遊走し，その数を増加させることが明らかとなり，特に感染モルモットにおいてその遊走集積が正常動物よりも著しく多かった．