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Research Note

Experimental Eosinophil Accumulation in Mice by a Thiol Protease from Metacercariae of *Paragonimus westermani*

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Eosinophil accumulation in tissue surrounding worms is a typical cellular response of the host associated with helminthic infections (Ansari and Williams, 1976; Warren *et al.*, 1967; Sugane and Oshima, 1982). Most recently it was noted that a thiol protease was secreted in the digestive tract of the metacercariae of *Paragonimus westermani*, and that the enzyme was excreted from the tract (Hamajima and Yamakami, 1985; Hamajima *et al.*, 1985). Therefore, we attempted to demonstrate whether the eosinophils are mobilized in peritoneal exudates following an injection of the enzyme into the peritoneal cavities of mice.

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). The enzyme was activated with 2.5 mM-cysteine in 50 mM-imidazole-HCl, pH 7.5, and was made isotonic with NaCl solution prior to the injection. Normal C57BL/6 male mice weighing 30-40 g and corresponding infected mice orally with 20 *P. westermani* metacercariae were used for the assay of *in vivo* eosinophil accumulations induced by the purified enzyme. Those normal and infected mice were divided into two groups; those in one group were given 0.2 ml of the intact enzyme (0.01 μg) intraperitoneally, while the mice in other group were injected with heat-inactivated enzyme (treatment at 80°C in a water bath for a few seconds) as the control.

All the mice were anaesthetized with either at 6, 12 and 18 hr after the injection. Peritoneal exudates were harvested using a total of 10 ml saline solution containing heparin (10 units/ml). Following light centrifugation, the supernatant was removed and the cells were resuspended in 1.0 ml of saline. Peripheral blood was obtained by severing the end of the tail. Eosinophils were stained with Discombe's diluting fluid (Discombe, 1946) and absolute eosinophil counts were performed in a Fuchs-Rosenthal chamber.

The data obtained were based on the mean ±SE of cell numbers of 6 mice in each experiment. Student's "t" test was used to assess the significance of difference between group means and P values <0.05 were considered significant. In normal and infected mice, eosinophil counts for peritoneal exudates and peripheral blood were made at 6, 12 and 18 hr after the enzyme injection.

Eosinophil accumulated in the peritoneal cavity following intraperitoneal injections of the protease (Fig. 1). In the normal mice, the number of eosinophils in the peritoneal cavities at 12 and 18 hr except for 6 hr following intraperitoneal injection of the enzyme exceeded those of the control ($P<0.05$-0.01).

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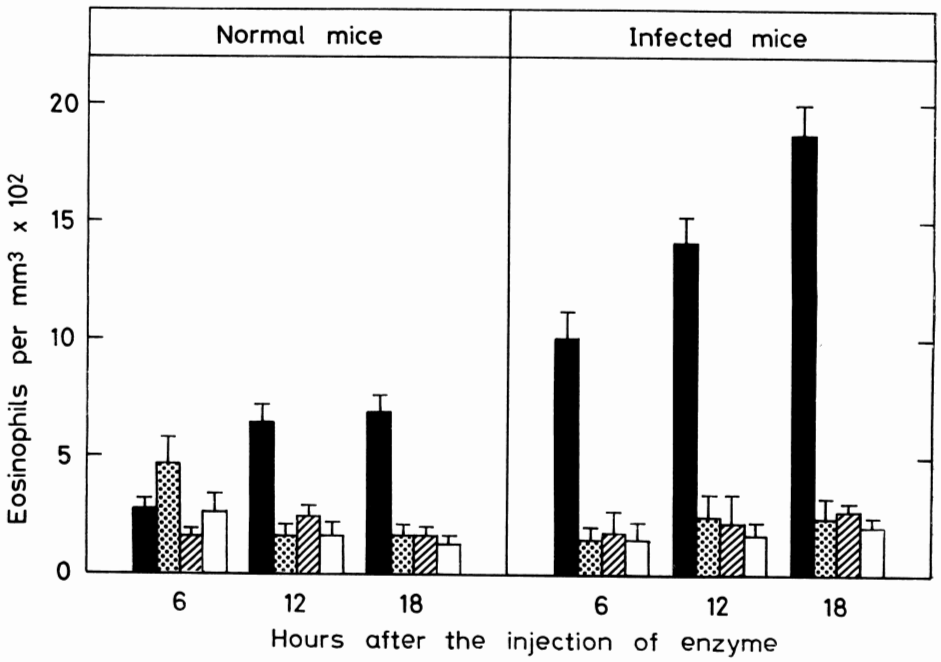

Fig. 1 Time-course of eosinophil accumulation in peritoneal exudates and peripheral blood of normal and infected mice after the intraperitoneal injection of thiol protease from *P. westermani* metacercariae. Results represent the mean±SE of cell numbers of 6 mice in each group: the number of peritoneal exudate cells in mice injected with the intact enzyme (■) or the heat-inactivated enzyme (▨); the number of peripheral blood cells after injection of intact enzyme (⁘) or the heat-inactivated one (□).

On the other hand, the number of peritoneal exudate eosinophils in the infected mice at 6, 12 and 18 hr post injection increased markedly over the control, respectively ($P<0.05$–0.01). While, the number of eosinophils in the peripheral blood 6, 12 and 18 hr after the injection did not exceed those of the control both in the normal and in the infected mice.

These results are similar to those reported for the effect of the injection of larval *Anisakis*, *Ascaris*, *Toxocara* and *Schistosoma* adults extracts on the eosinophil accumulations in normal guinea pigs and in mice infected with *T. canis* by Tanaka and Toris (1978), Sugane and Oshima (1980) and Horii *et al.* (1984). Thus, this suggests that this eosinophil accumulation was mediated by the enzyme, and/or some liberated peptides from host tissue proteins hydrolyzed with the enzyme as reported previously by Hamajima and Yamakami (1985). The intraperitoneal injection of the enzyme into infected mice resulted in a high peritoneal eosinophils as compared with that from normal mice. An identical result was obtained by Sugane and Oshima (1980) for the recovery of large numbers of eosinophils from mice infected with *T. canis*. It seems that this high intraperitoneal cell migration in infected mice may probably be reinforced by a release of lymphocyte-derived eosinophil chemotactic factor.

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短　報

ウェステルマン肺吸虫メタセルカリアの蛋白水解酵素によるマウス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より蛋白水解酵素を抽出し，その精製酵素を正常および本メタセルカリア経口感染マウスの腹腔に注射し，そこにおける好酸球数の経時的変化を検討した．その結果，本酵素注射は腹腔内に好酸球を遊走し，集積させることが明らかとなり，特に感染マウスにおいてその集積が多かった．