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Research Note

Inhibition of Acid Hemoglobin Protease of a Lung Fluke by Some Human Immunoglobulins

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An acid hemoglobin protease that hydrolyzes host hemoglobin has been found in some *Paragonimus* worms (Hamajima and Yamagami, 1981; Yamagami and Hamajima, 1981; Yamagami and Hamajima, 1982). The protease activity was inhibited by sera from some mammalian hosts and antiproteases of human plasma. On the other hand, hydrolysis of the hemoglobin by the enzyme was stimulated by C3 component (Hamajima, Yamakami and Oguma, 1982). Thus, we have been interested in studying the influence of some immunoglobulins on the hydrolysis of hemoglobin by the enzyme from *Paragonimus westermani* in connection with the host-parasite relationship. Therefore, the present experiment was carried out to determine whether the formation of the hydrolysates of human hemoglobin by purified enzyme is appreciably inhibited by human immunoglobulin A, M and G.

P. westermani (triploid type) adult worms were removed from worm cysts of the lungs of dogs 10 months after inoculation with metacercariae isolated from *Eriocheir japonicus* collected on Tsushima Is., Japan. The specimens were rinsed with saline solution before preparation. Human immunoglobulin A, M and G were supplied by the Behring Institute via Hoechst Japan. α_1-Antitrypsin for affinity chromatography was purchased from Sigma Chemical Co. Sepharose 4B, DEAE-cellulose and CM-cellulose were obtained from Pharmacia Fine Chemicals, Whatman Biochemicals and Serva Feinbiochemica GmbH, respectively. All chemicals employed were of the highest purity. Hemoglobin protease of the lung fluke was purified by affinity chromatography (Hamajima and Yamagami, 1981). Protein concentration was determined by the method of Lowry *et al.* (1951) with bovine serum albumin as a standard. Assay of proteolytic activity was basically performed according to the method reported by Anson (1939). Purified enzyme (2.33 units per assay mixture) was used in the experiment. The enzyme (20 μl) was treated with 10 μl of 0.05 M phosphate buffer, pH 6.0 in the presence or absence of immunoglobulin (1.85×10^{-10} moles) for 30 minutes at 0 C, and then 75 μl of hemoglobin (500 μg) in 0.2 M acetate buffer, pH 4.0, (final volume, 105 μl) were added to the previous mixture. The reaction mixture was incubated for 40 minutes at 37 C. Then, trichloroacetic acid was added to a final concentration of 2.38%. The mixture was allowed to stand for 60 minutes at 0 C, and then centrifuged.

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Table 1 Inhibition of purified acid hemoglobin protease from the adult *P. westermani* by some human immunoglobulins

Protein	ΔOD ($\times 10^{-3}$) at 660 nm Mean±SD	Inhibition (%)	P
Control	186±5.7	0	—
Immunoglobulin A	131±2.6	30	<0.001
Immunoglobulin M	0±0.0	100	<0.001
Immunoglobulin G	88±5.9	53	<0.001

Each value represents the mean of triplicate determinations with standard deviations (SD). Probability (P) is expressed in P-value.

The liberated peptide in the supernatant was measured by the methods of Lowry *et al.* (1951) at 660 nm with a Beckman spectrophotometer. Evaluation of the inhibition of the protease by immunoglobulins was based on decrease in the hydrolysates from the substrate as compared with control. The activity of the enzyme was expressed as ΔOD at 660 nm. One unit of the enzyme activity was defined as the activity producing an increase of 1.0 in absorbance at 660 nm per minute per ml.

Table 1 shows the inhibition of the purified enzyme by human immunoglobulin A, M and G. The extent of hydrolysis of hemoglobin by the enzyme was significantly inhibited below the level of the control when each of immunoglobulins was added as an inhibitor. In particular, the addition of the immunoglobulin M produced a complete inhibition of the hydrolysate formation. However, the immunoglobulin in the absence of the enzyme did not decrease the hydrolysates below the level of a blank reaction system in which both the enzyme and the immunoglobulins were absent.

Lung flukes injure various tissues and organs when penetrating and feeding during their migrations to the host lungs (Yokogawa *et al.*, 1960; Yokogawa, *et al.*, 1962). In addition, the enzyme hydrolyzes host hemoglobin for nutrition of the flukes. Moreover, human body produces several immunoglobulins. Therefore, inhibition of the enzyme in the fluks by some immunoglobulins is of great interest from the standpoint of the protection of the host against the flukes. In the present study, hydrolysis of hemoglobin by the enzyme was inhibited by some immunoglobulins. Identical results were obtained from the studies on the inhibitory effects of plasma antiproteases on cercarial protease of *Schistosoma mansoni* (Asch and Dresden, 1977) and hemoglobin protease of the lung flukes (Hamajima and Yamagami, 1981). From these results, it was concluded that the flukes did not sufficiently ingest hemoglobin for nutrition, because of the inhibitory action of some antiproteases and immunoglobulins against the protease that hydrolyzes hemoglobin. Thus, it seems probable that these immunoglobulins are effective for host protection against the worms in concert with some antiproteases.

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短　　報

人 immunoglobulins による肺吸虫ヘモグロビン分解酵素の阻害作用

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ウエステルマン肺吸虫 (3n 型) の終宿主における寄生現象を生化学面から解明するため，本肺吸虫成虫におけるヘモグロビン分解酵素による人ヘモグロビンの水解に対する人 immunoglobulin A, M および G の作用を検討した．その結果，これらの immunoglobulins は本酵素によるヘモグロビンの水解を阻害した．このことから immunoglobulins は，肺吸虫の宿主での栄養摂取を防げ，宿主の本吸虫に対する防御作用に重要な関係をもっているものと考えられる．