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☐ **Brief Communication** ☐

Activities of different cysteine proteases of *Paragonimus westermani* in cleaving human IgG

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Abstract: Cleaving host immunoglobulins is a well known mechanism of evading host immune reactions exploited by helminth parasites. Secreted cysteine proteases of helminth are a part of enzymes cleaving host IgG. *Paragonimus westermani* produces at least six different species of the cysteine protease in its developmental stages. This study was undertaken to evaluate comparatively the activities against human IgG by the different enzymes. When the metacercariae, which secrete 27 and 28 kDa cysteine proteases, were incubated in human IgG solution, IgG was degraded at its hinge region. Further incubation resulted complete hydrolysis. From 4-week and 7-week old juveniles and 16-week old adults of *P. westermani*, five different enzymes at 15, 17, 27, 28 and 53 kDa have been purified, if the enzyme with the same molecular mass is regarded to be identical. In cleaving human IgG, each cysteine protease exhibited decreasing activities with age.

Key words: *Paragonimus westermani*, cysteine protease, immune evasion, IgG cleavage

Since the neutral thiol protease (27 kDa) of *P. westermani* metacercariae were purified (Yamakami and Hamajima, 1987; Yamamoto *et al.*, 1994), at least six species of the cysteine proteases have been described. In addition to the 27 kDa metacercarial enzyme, 17 kDa cysteine protease of adult (Song and Kim, 1994), 28 kDa metacercarial enzyme (Chung *et al.*, 1995), 34 kDa from eggs (Kang *et al.*, 1995), 15 and 53 kDa enzymes from juveniles and adults (Chung, 1996) have been purified. The diverse biological roles of the enzymes include metacercarial excystment (Chung *et al.*, 1995), nutrient uptake and worm migration because the enzymes degrade hemoglobin and extracellular matrices such as collagen and fibronectin. Moreover, of many host immune evasion mechanisms of helminth parasites (Maizels *et al.*, 1993), degrading host IgG is an important strategy which is executed by different cysteine proteases (Kong *et al.*, 1994). In order to characterize the activities of different cysteine proteases of *P. westermani*, cleavage of human IgG molecule was observed.

Collection of the metacercariae of *P. westermani* from naturally infected crayfish, experimental infections to cats and dogs, and harvesting 4-week and 7-week old juveniles and 16-week old adults were described

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elsewhere (Chung, 1996). From crude extracts of the metacercariae, juveniles and adults, 17 proteins with cysteine protease activities were purified by a series of chromatographies. The purity of each enzyme was confirmed by a single band each in SDS-PAGE analysis (Chung, 1996).

The hydrolyzing activity of the purified enzymes was observed using human IgG as a substrate (Kong *et al.*, 1994). Human IgG was purified by Protein A-Sepharose CL-4B affinity chromatography (Pharmacia-LKB, Piscataway, NJ, USA). Twenty excysted metacercariae were incubated in 2.5 ml IgG solution (3 mg/ml in 10 mM phosphate buffered saline, pH 7.4) in the presence of 1 mM dithiothreitol (DTT) for 1, 3 and 10 hours at 37°C, respectively. The reactions were stopped by adding iodoacetamide (1 mM in final concentration). The reactants were analysed by immunoblot using peroxidase-conjugated anti-human Fab (Cappel, Durham, NC, USA).

IgG cleaving activity was observed in a reaction mixture, consisted of 5 μl of the each purified enzyme (0.5 μg protein), 2 μl dithiothreitol (2 mM), 10 μl IgG (20 μg) and 20 μl sodium acetate buffer (0.1 M, pH 6.4), which were incubated for 30 minutes or 1 hour at 37°C. Reactions were terminated by adding 10 μl iodoacetamide. Reaction mixtures were centrifuged at 20,000 rpm for 10 minutes. The supernatant fractions were subjected to SDS-PAGE and analyzed with Coomassie blue staining.

Degradation of IgG by the live metacercarial secretion are shown in Fig. 1A. With incubation time, recognitions at heavy chain (50 kDa) by anti-Fab became fainter. Light chain at 26 kDa does not change its reactivity until the 3 hours incubation. A degradation product at 32 kDa (Fab fragment) appears in 1- and 3-hour reactants. In the 10-hour reactant, heavy and light chains disappeared completely as well as Fab fragment.

The cysteine proteases at 15, 17, 27, 28 and 53 kDa, purified from each worm of different stages, were reacted with IgG. Reactants are analysed by SDS-PAGE (Fig. 1B). Three different degradation fragments of IgG are shown at 10, 15 kDa and 32 kDa (Fab). Faint Fab fragment (32 kDa) are exhibited in 15 and 17 kDa enzymes from 16-week adults, 53 kDa enzyme of 7-week juveniles and 16-week adults. Digestion fragments below 20 kDa are shown in metacercarial incubation products, and reactants with 17 kDa enzyme from juveniles.

IgG degradation by the 28 kDa enzyme from different ages are shown in Fig 1C. In 30 minutes incubation with the cysteine proteases from worms of different age, the 28 kDa enzyme produce less amounts of 32 and 10 kDa fragments while heavy chain is less affected and light chain remains unaffected.

In this study, we described the proteolytic cleavage of IgG either by live worms or by purified enzymes from different stages of *P. westermani*. In cleaving IgG, those purified from younger worms show higher activities than those from elders (Fig. 1C). This result is coincided with the decreasing specific activities of cysteine proteases which were purified from different maturation stages (Song and Dresden, 1990). The changing specific activity of cysteine proteases were mainly due to decreased activity of the metacercarial enzymes at 27 and 28 kDa (Chung, 1996).

The secreted metacercarial cysteine proteases of *P. westermani* reveal higher activities in cleaving IgG molecules than any cysteine protease of juvenile and adult worms (Fig. 1C). As also shown in Fig. 1A, they cleave IgG heavy chain into Fab and 10 kDa fragments. Prolonged incubation with the live metacercariae up to 10 hours hydrolyzed IgG completely which are not detected in SDS-PAGE analysis. This result suggests that the metacercarial enzymes may have other specific active sites in cleaving the heavy chain molecules.

As Hamajima and colleagues (1986) described, the metacercarial cysteine protease accumulated eosinophils when injected into mouse peritoneum. Carmona *et al.* (1993) reported also that newly excysted juveniles of *Fasciola hepatica* secrete cathepsin L which prevented eosinophil attachment to the body surface of the juveniles. All these findings suggested that the high activities of metacercarial cysteine proteases were related with high eosinophilia in the early stage of paragonimiasis. Decreasing levels of peripheral

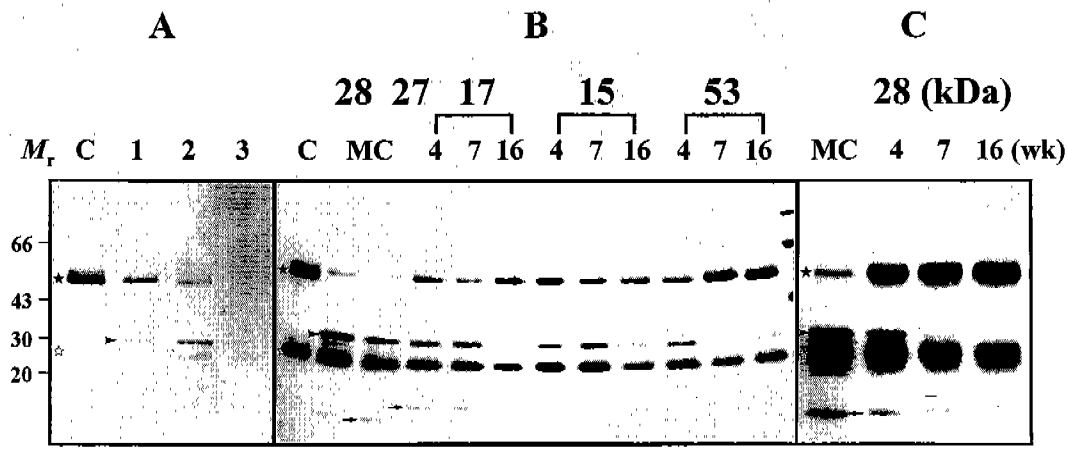

Fig. 1. (A) Immunoblot analysis of IgG cleavage. Twenty, newly excysted live metacercariae were incubated in human IgG solution (3 mg/ml) for 1 hour (lane 1), 3 hours (lane 2) and 10 hours (lane 3) at 37°C. The reactants were separated by 7.5-15% SDS-PAGE, transferblotted to PVDF membrane and probed with anti-human Fab. A degradation product at 32 kDa (▶) was detected. Ten hour incubation exhibited complete hydrolysis of the reacted IgG. Heavy (★) and light chain (☆) of IgG. M_r, molecular weight in kDa; C, control IgG. (B) Degradation of human IgG by purified cysteine proteases from 4 different stages of *P. westermani*. The reaction mixtures were incubated for 2 hour at 37°C and separated in 7.5-15% SDS-PAGE and Coomassie stained. Digestion products at 32 (▶), 15 (⇢) and 10 (⇢) kDa were detected. Numbers at the top of the figure indicated the M_r of the purified enzymes and numbers in the second row, the age of *P. westermani* in weeks. C, control IgG; MC, metacercariae. (C) Cleavage of IgG by the 28 kDa cysteine proteases purified from different maturation stages. The reaction mixtures were incubated for 30 minutes at 37°C and resolved by 7.5-15% SDS-PAGE, then Coomassie stained. Markings are the same as described in (B).

eosinophilia in chronic paragonimiasis can be studied in relation with decreasing activities of cysteine proteases, especially against IgG molecules.

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=초록=

발육단계별로 정제한 폐흡충 시스테인계열 단백분해효소의 IgG 분해양상

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폐흡충 피낭유충, 4주, 7주 및 16주 충체에서 분자량 15, 17, 27, 28 및 53 kDa인 시스테인 단백질 분해효소를 정제하고 각 효소가 IgG를 분해하는 양상을 비교하였고, 탈낭시킨 폐흡충 피낭유충을 IgG 용액에 배양하여 유충이 분비하는 27 및 28 kDa 단백질분해효소가 IgG을 분해하는 양상을 관찰하였다. 1시간 배양하였을 때 IgG heavy chain은 Fab로 분해되었으며, 10시간 반응시킨 결과 전체 IgG 분자가 분해되었다. 피낭유충에서 정제한 27 및 28 kDa 효소, 4주, 7주(성장충) 충체 및 16주 성충에서 정제한 15, 17, 27, 28, 53 kDa 효소와 IgG를 반응시킨 결과 모든 효소가 IgG를 hinge region에서 분해하였다. IgG 분해 양상은 피낭유충에서 가장 강하고, 성충으로 성장하면서 각 효소의 IgG 분해능이 점차 감소하는 양상을 보였다.

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