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Antibody-Dependent Adherence of Guinea Pig Eosinophils to the Third-stage Larvae of *Metastrongylus apri*

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ABSTRACT. The serum of guinea pigs infected with *Metastrongylus apri* (*M. apri*) promoted the adherence of peritoneal eosinophils, obtained from normal and infected animals, to the third-stage larvae *in vitro*. The promotion of eosinophil adherence was first detected in the serum 14 days after infection followed by marked increase in the activity as the infection progresses. In such sera, immunoglobulin G capable of reacting with the cuticular surface of the larvae was demonstrated. The antibody-dependent eosinophil adherence reduced the infectivity of the larvae, suggesting that eosinophils may play a protective role against *M. apri* *in vivo*.—**KEY WORDS:** eosinophil, guinea pig, *Metastrongylus apri*.

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It is well known that eosinophils act as effector cells for antibody-dependent cytotoxicity against *Schistosoma mansoni* schistosomula [2], newborn larvae of *Trichinella spiralis* [10, 17], microfilariae of *Dipetalonema viteae* [6], the young adult worms of *Angiostrongylus cantonensis* [24], *Strongyloides ratti* [22] and haptenated schistosomula of *S. japonicum* [12] *in vitro*. Furthermore, anti-eosinophil serum has been shown to diminish immune protection against *S. mansoni* and *T. spiralis* [15, 5]. The protective role of eosinophils in *Metastrongylus apri* infection, however, has not been reported, although it is well established that systemic or local eosinophilia occurs in naturally infected pigs and in experimentally infected guinea pigs [14, 18]. The aim of the present work was to assess a possible protective role of eosinophils against infection with the third-stage larvae of *M. apri* in guinea pigs.

The third-stage larvae were prepared by digesting earth-worms (*Eisenia foetida*) experimentally infected with *M. apri* eggs. They were collected by the floating gel meth-

od [20], and suspended in a culture medium, RPMI 1640, containing 20% fetal calf serum (FCS) inactivated by heating at 56°C for 30 min [7]. Sera were obtained from normal male Hartley guinea pigs weighing 250 to 350 g and from those orally infected with 50 third-stage larvae at appropriate intervals (see Fig. 2). Sera were inactivated by heating at 56°C for 30 min.

Eosinophil-rich peritoneal exudate cells were collected by peritoneal lavage with RPMI 1640 [19] from uninfected guinea pigs, which had received repeated injections of horse serum, and also from *M. apri* infected guinea pigs at 3 weeks of infection 48 hr before the cell harvest, these animals received an intraperitoneal injection of 10 ml of 10% proteose peptone [21]. The cells were suspended in RPMI 1640 medium supplemented with 10% heat-inactivated FCS and antibiotics (penicillin 100 units/ml and streptomycin 100 µg/ml) and incubated in plastic tissue culture dishes for 2 hr at 37°C under 5% CO₂ atmosphere. Non-adherent cells were subsequently collected, washed twice

Fig. 1. Time course of the adherence of peritoneal eosinophil rich cells from (A) normal or (B) *M. apri*-infected guinea pigs to the third-stage larvae in the presence of serum from guinea pigs infected with *M. apri*. Cell adherence score: 4+, whole larval surface was covered with PEC; 3+, 60–90%; 2+, 30–60%; 1+, 10–30%; 0, less than 10% of the surface was covered. ○, eosinophil-rich peritoneal exudate cells from normal guinea pigs (n-PEC) plus infected serum (N=25). Mean±SD; △, n-PEC plus normal guinea pig serum (NGS) (N=20); □, n-PEC plus medium (N=20); ●, eosinophil-rich peritoneal cells from *M. apri* infected guinea pigs at 3 weeks of infection (i-PEC) plus infected serum (N=25); ▲, i-PEC plus NGS (N=20); ■, i-PEC plus medium (N=20). Statistical differences: ○ vs △ & □ = $p < 0.01$; ● vs ▲ & ■ = $p < 0.01$.

and re-suspended in the medium. Peritoneal exudate cells (PEC) thus prepared from normal or infected animals consisted of 49% to 60% eosinophils, 36% to 22% macrophages, 9% to 4% lymphocytes and 6% to 14% neutrophils.

The cell adherence assay to the third-stage larvae was carried out in a microslide culture chamber (Bellco Glass, Vineland, N. J.) with the following sequence. Thirty to forty larvae in 0.05 ml of RPMI 1640 medium containing 20% FCS were dispensed in each well of the chamber, followed by the addition of 0.05 ml of the normal, infected serum (diluted 1:12) or RPMI medium, and finally 0.05 ml of normal or infected guinea pig's PEC was added to make a ratio of PEC:larvae=20.000:1 [24]. After an appropriate time of

Fig. 2. Cell adherence-promoting effect of sera obtained at varying times from guinea pigs infected with *M. apri*. Cell (n-PEC) adherence score denotes mean ± SD at 2 hr of incubation. Shaded area shows mean ± SD for larvae, incubated with n-PEC plus NGS. See Fig. 1 for abbreviations. Differences between the scores from incubations with infected serum and with NGS were significant at the levels of 5% (*) and 1% (**).

incubation at 37°C under 5% CO₂ atmosphere, samples were removed from each well, washed twice with PBS and fixed with methyl alcohol to be stained with Giemsa. The interaction of PEC and larvae was estimated by a light microscope and adherence was scored according to criteria described by Yoshimura (see legend in Fig. 1) [24].

Figure 1 shows the time course of the adherence of PEC to the larvae incubated with normal or infected guinea pig serum (obtained at 5 weeks after infection) or without serum. Visible adherence was seen only when the larvae was incubated with infected guinea pig serum. The cell adherence-promoting effect of the infected serum was first detected at 2 weeks after larval inoculation and increased as the infection progressed (Fig. 2). Figure 3 shows the interaction between IgG antibody from the infected guinea pig serum and larval surface. These

Fig. 3. Immuno-fluorescence examination of *M. apri* third-stage larvae sensitized with normal and infected guinea pig serum. The larvae were stained with FITC-conjugated IgG of rabbit anti-guinea pig IgG (Miles-Yeda LTD, Rehovot, Israel) (diluted 1:80) after they were incubated for 30 min with (A) normal serum (diluted 1:30) or (B) *M. apri* infected guinea pig serum obtained at 5 weeks of infection (diluted 1:30). A, B: $\times 100$.

Fig. 4. Giemsa stained preparation showing cells adhering to the surface of *M. apri* third-stage larvae. About 91% of the cells are eosinophils (adherence score: +3). A: $\times 200$, B: $\times 800$.

Table 1. Differential counts of peritoneal cells adhering to the third-stage larvae of *M. apri*.

Third-stage larvae ^{a)} incubated with	Cells adhered (mean $\pm$ SD) (N=20)		
	Eosinophil	Macrophage	Neutrophil
n-PEC+IGS ^{b)}	91.9 $\pm$ 3.8	6.9 $\pm$ 3.2	1.2 $\pm$ 1.5
i-PEC+IGS	91.6 $\pm$ 2.1	8.0 $\pm$ 2.0	0.3 $\pm$ 0.3

a) Obtained 2 hr after incubation, and stained with Giemsa.

b) Obtained at 5 weeks of infection.

results indicate that IgG antibody against the larvae is possibly associated with the cell adherence reaction. In addition, the adherence score of normal PEC rose gradually up

to 48 hr of incubation, but the score of PEC from infected animals rose rapidly to a plateau level at 2 hr of incubation. The PEC of infected animals might have been sensitized

Table 2. Effects of eosinophil-rich peritoneal exudate cells on the infectivity of the third-stage larvae of *M. apri*

Third-stage larvae ^{a)} incubated with	No. of animals infected	% Worm recovery ^{c)} mean±SD
n-PEC+IGS ^{b)}	7	7.7± 4.4
i-PEC+IGS	7	7.6± 5.8
IGS	7	12.9± 5.1
n-PEC+RPMI 1640	6	36.4±10.9 ^{d)}
i-PEC+RPMI 1640	7	36.0± 8.7 ^{d)}

a, b) See the foot-notes on Table 1.

c) From two independent experiments.

d) Statistical difference vs other data ($P<0.01$).

with the particular antibody *in vivo*. Differential counts of leukocytes indicated that the majority (about 91%) of the cells adhering to the larvae were eosinophils, but a small number of macrophages and neutrophils were also included (Table 1 and Fig. 4).

Subsequently, we attempted to determine the effect of PEC adherence on the larval infectivity. The third-stage larvae previously incubated for 16 hr with both PEC and infected guinea pig serum, or without PEC or the serum, were collected, washed, adjusted for their concentrations and then inoculated orally with 60 larvae per a guinea pig. Three weeks later, the animals were sacrificed to determine the worms invading the lungs. Table 2 shows that the worm recovery rate from the lungs was significantly less in the animals inoculated with the larvae previously incubated with PEC from either normal or infected animals plus infected serum than in those inoculated with larvae incubated with PEC or serum alone.

The current studies clearly indicate that the eosinophil adherence to *M. apri* larvae was dependent on the specific IgG antibody and this adherence reduced the larval infectivity. Although the mechanism of this reduction in infectivity remains to be determined, the helminthocidal activity of eosinophils has been reported to be due to granular major basic protein [3, 23], eosinophil cationic protein [9, 16], eosinophil peroxidase-H₂O₂-halide system [4, 8], phospholipase D [13], hydro-

gen peroxide [1, 11] and superoxide radical [1] *in vitro*. It should also be mentioned that the larvae of *M. apri* incubated with the infected guinea pig serum alone showed a slight reduction in infectivity (Table 2), suggesting the detrimental effect of the antibody itself. In addition, the role of macrophages adhering to the cuticular surface of *M. apri* third-stage larvae (Table 1) has yet to be determined.

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REFERENCES

1. Bass, D. A., Szejda, P., DeChatelet, L. R., and McCall, C. E. 1979. *Fed. Proc.* 38 (1077): 4493.
2. Butterworth, A. E., Sturrock, R. F., Houba, V., Mahmoud, A. A. F., Sher, A., and Ress, P. H. 1975. *Nature* 256: 727-729.
3. Butterworth, A. E., Wassom, D. L., Gleich, G. J., Loegering, D. A., and David, J. R. 1979. *J. Immunol.* 122: 221-229.
4. Buys, J., Weber, R., van Stigt, R., and Ruitenberg, E. J. 1981. *Eur. J. Immunol.* 11: 843-845.
5. Grove, D. L., Mahmoud, A. A. F., and Warren, K. S. 1977. *J. Exp. Med.* 145: 755-759.
6. Haque, A., Ouaisi, A., Joseph, M., Capron, M., and Capron, A. 1981. *J. Immunol.* 127: 716-725.
7. Hata, H., Fujita, J., and Yasuraoka, K. 1980. *Jpn. J. Exp. Med.* 50: 365-374.
8. Jong, E. C., Mahmoud, A. A. F., and Klebanoff, S. J. 1981. *J. Immunol.* 126: 468-471.
9. Kay, A. B. 1979. *J. Allergy Clin. Immunol.* 64: 90-104.
10. Kazura, J. W., and Aikawa, M. 1980. *J. Immu-*

- nol.* 124: 355–361.
11. Kazura, J. W., Fanning, M. M., Blumer, J. L., and Mahmoud, A. A. F. 1981. *J. Clin. Invest.* 67: 93–102.
 12. Kojima, S., Yamamoto, N., Kanazawa, T., and Ovary, Z. 1985. *J. Immunol.* 134: 2719–2722.
 13. Lempereur, C., Capron, M., and Capron, A. 1980. *J. Immunol. Methods.* 33: 249–260.
 14. Mackenzie, A. 1959. *Vet. Rec.* 71: 209–214.
 15. Mahmoud, A. A. F., Warren, K. S., and Peters, P. A. 1975. *J. Exp. Med.* 142: 805–813.
 16. McLaren, D. J., McKean, J. R., Olsson, I., Venge, P., and Kay, A. B. 1981. *Parasite Immunol.* 3: 359–373.
 17. Ortega-Pierres, G., Mackenzie, C. D., and Parkhouse, R. M. E. 1984. *Parasite Immunol.* 6: 275–284.
 18. Sasaki, O., and Hyogo, Y. 1977. *Bull. Akita Agric. Coll.* 3: 11–21 (in Japanese with English summary).
 19. Sasaki, O., and Katsuno, M. 1982. *Jpn. J. Vet. Sci.* 44: 787–793.
 20. Sasaki, O., and Katsuno, M. 1983. *Jpn. J. Vet. Sci.* 45: 807–809.
 21. Sugane, K., and Oshima, T. 1980. *Am. J. Trop. Med. Hyg.* 29: 799–802.
 22. Tam, N. D., Papadimitriou, J., and Keast, D. 1983. *Aust. J. Exp. Biol. Med. Sci.* 61: 629–636.
 23. Wassom, D. L., and Gleich, G. J. 1979. *Am. J. Trop. Med. Hyg.* 28: 860–863.
 24. Yoshimura, K., Uchida, K., Sato, K., and Oya, H. 1983. *Jpn. J. Parasit.* 32: 489–500.

要 約

正常ならびに豚肺虫感染モルモット好酸球の豚肺虫 3 期子虫への抗体依存性附着とその感染力に及ぼす効果 (短報) : 佐々木 脩・勝野正則¹⁾ (秋田県立農業短期大学家畜衛生学教室, ¹⁾東北大学農学部家畜衛生学教室) ———感染後14日以降の豚肺虫感染モルモットの血清中には正常モルモットならびに豚肺虫感染モルモット由来の好酸球を 3 期子虫へ附着させる作用が認められ, その作用は経時的に増強した。この好酸球附着現象は子虫表面に存在する抗原に反応する免疫グロブリン G を介しておこり, この抗体依存性好酸球の附着は, 3 期子虫の感染力の低下をもたらした。これらの成績から好酸球は宿主体内で感染防御に関与するものと推測された。