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Research Note

Antibody against the cuticle surface of *Trichinella spiralis* muscle larvae predominantly reacts to parasite constituents of pI 4.0 and 5.4

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The surface antigens of *Trichinella spiralis* show stage specificity (Mackenzie *et al.*, 1978; Philipp *et al.*, 1980, 1981; Parkhouse *et al.*, 1981; Jungery *et al.*, 1983; Almond *et al.*, 1986; McLaren *et al.*, 1987) and species specificity (Parkhouse *et al.*, 1981), and induce protective immunity to the host (Grencis *et al.*, 1986), however, the origin and immunochemical properties of the cuticle surface antigens are largely unknown. In the previous study we revealed that antibody against the cuticle surface of *T. spiralis* muscle larvae recognizes stichocyte α granules and the esophagus occupying substance (EOS) as well as the cuticle surface (Takahashi *et al.*, 1990b). The purpose of this study is to identify the isoelectric point of antigen molecules which react to anti-cuticle surface antibody by the combination of isoelectric focusing and immunoblotting for better understanding of the cuticle surface.

Soluble antigen of muscle larvae was prepared as follow; the muscle tissue of *T. spiralis* infected mice was minced and then digested in 0.5% pepsin-0.2N HCl solution at 37°C for 3hr. The recovered larvae were washed several times with phosphate buffered saline (PBS), ultrasonicated (20kHz, TOMY SEIKO Co., Ltd. Tokyo, Japan) at 4°C for 20 min and incubated in PBS at 4°C overnight. After centrifuging at 10,000 rpm for 20 min at 4°C, the supernatant was dialysed against distilled water and lyophilized. The PBS soluble antigen thus obtained was processed for isoelectric focusing (IEF) on thin layer polyacrylamide gel of the pH range of 3.5–9.5 (LKB Ampholine PAG plate No. 1804-101 Bromma, Sweden). Ten μg of the antigen was loaded on each track and electrophoresed at constant current of 6mA at 4°C for 4 hr.

After IEF separation, the antigen was electrotransferred to a polyvinyliden difluolide (PVDF) membrane (NIHON MILIPORE KOGYO K.K. Yonezawa, Japan) and the membrane was cut into strips. The strips were then incubated in 3% BSA-PBS at 37°C for 1 hr to block nonspecific binding. After extensive washings with 0.05% Tween 20 in PBS, the PVDF strips were incubated with the followings; first, anti-cuticle surface antibody which was obtained by immunoaffinity chromatography using chemically-fixed muscle larvae as the solid-phase antigen (Takahashi *et al.*, 1990b); second, sera from infected Fischer rats; third, normal rat sera. The incubation was performed for 1hr at 37°C. The strips were washed again with 0.05% Tween 20

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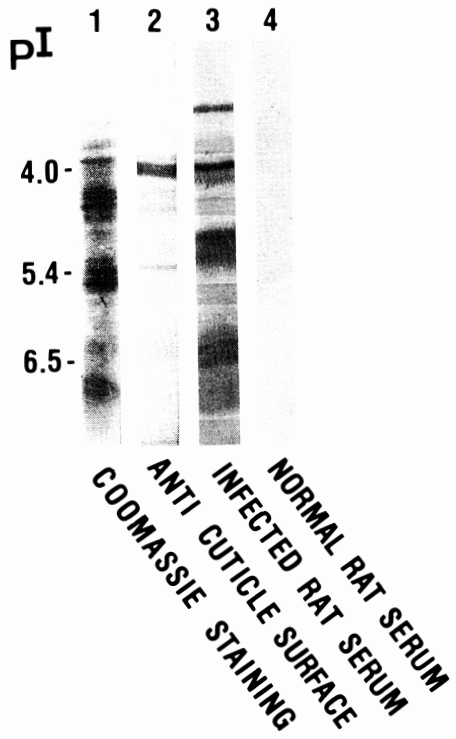
in PBS and incubated with the peroxidase-conjugated anti rat IgG (Capel USA) diluted at 1:300 in 3% BSA-PBS. After washing with 0.05M tris HCl buffer (pH 7.6), the strips were incubated in a reaction mixture (3mg of 3,3-diaminobenzidine and 10 μl of H_2O_2 in 10ml of 0.05M tris HCl buffer, pH 7.6).

PBS soluble antigen of *T. spiralis* muscle larvae was composed of at least eighteen bands that can be visualized by coomassie brilliant blue R-250 staining in the range of pH 3.5–9.5 (Figure, lane 1). By immunoblot analysis, the infected rat sera reacted to several bands of pI 3.7, 4.0, 4.1, 4.4, 4.9~5.4 and 6.3~6.5 (Figure, lane 3), showing that a wide variety of antigens were recognized by rats during a primary infection. On the other hand, immunoaffinity-purified anti-cuticle surface antibody strongly reacted to only two bands, pI 4.0 and 5.4 (Figure, lane 2). Although it also reacted to the bands of pI 3.9, 4.4 and 4.7, the intensity was much less weak. Normal rat sera did not react to any bands (Figure, lane 4).

Since the cuticle surface shares same antigenicity with stichocyte α granules and the EOS (Takahashi *et al.* 1990b), our immunoblotting analysis raises the possibility that pI 4.0 and 5.4 antigens may be derived from these structures. The assumption that pI 4.0 antigen is derived from the cuticle surface, α granules and/or the EOS does not contradict with our previous studies on the time course of antibody response. Because antibodies against pI 4.0 antigen are detected in a relatively late phase of *T. spiralis* infection (Mizuno *et al.* 1989), and so are antibodies against the cuticle surface, α granules and the EOS (slow responding group, Takahashi *et al.*, 1990a). The pI 5.4 antigen, however, cannot readily be concluded to be of the cuticle surface, α granules and/or the EOS origin. We have revealed that pI 5.4 antigen is a rapid responding group antigen whose antibody can be detected in a early phase of the infection (Mizuno *et al.* 1989). If pI 5.4 antigen is derived from the cuticle surface, the cuticle surface has to be a rapid responding group antigen, which is not the case. The surface is a slow responding group antigen (Takahashi *et al.*, 1990a).



Western immunoblot analysis of PBS soluble antigen of *T.spiralis* muscle larva recognized by immunoaffinity-purified anti-cuticle surface antibody (lane 2), infected rat serum (lane 3) or normal rat serum (lane 4).
lane 1; PBS soluble antigen of *T. spiralis* transferred to PVDF membrane was stained with Coomassie brilliant blue R-250.

Although much still remains to be explained about pI 5.4 antigen, the present observation that the anti-cuticle surface antibody reacted to the epitope on moieties of pI 4.0 leads to a useful suggestion; that is the cuticle surface antigen of immunological importance may be extracted from the band pI 4.0 which is endowed with strongest antigenicity (Despommier 1981). Since the advantage of IEF is that proteins can be separated without major degeneration, the pI 4.0 band may provide a practical source of cuticle surface antigen for the use in immunological studies including an immunodiagnostic test of the disease and a protection mechanism against oncoming infection.

References

1) Almond, N. M., McLaren, D. J. and Parkhouse, R. M. E. (1986): A comparison of the surface and secretions of *Trichinella pseudospiralis* and *T. spiralis*. Parasitology, 93, 163–176.
2) Despommier, D. D. (1981): Partial purification and characterization of protection-inducing antigens from the muscle larvae of *Trichinella spiralis* by molecular sizing chromatography and preparative flat bed isoelectric focusing. Parasit. Immunol., 3, 261–272.
3) Grencis, R. K., Crawford, C., Pritchard, D. I., Behnke, J. M. and Wakelin, D. (1986): Immunization of mice with surface antigens from the muscle larvae of *Trichinella spiralis*. Parasit. Immunol., 8, 587–596.
4) Jungery, M., Clark, N. W. T. and Parkhouse, R. M. E. (1983): A major change in surface antigens during the maturation of newborn larvae of *Trichinella spiralis*. Mol. Biochem. Parasitol., 7, 101–109.
5) Mackenzie, C. D., Preston, P. M. and Ogilvie, B. M. (1978): Immunological properties of the surface of parasitic nematodes. Nature, 276, 826–828.
6) McLaren, D. J., Ortega-Pierres, G. and Parkhouse, R. M. E. (1987): *Trichinella spiralis*: immunocytochemical localization of surface and intracellular antigens using monoclonal antibody probes. Parasitology, 94, 101–114.
7) Mizuno, N., Nishiyama, T., Uno, T., Takahashi, Y. and Araki, T. (1989): Antigenicity of *Trichinella spiralis* (9). Jpn. J. Parasitol., 38, 22A.
8) Parkhouse, R. M. E., Philipp, M. and Ogilvie, B. M. (1981): Characterization of surface antigens of *Trichinella spiralis* infective larvae. Parasit. Immunol., 3, 339–352.
9) Philipp, M., Parkhouse, R. M. E. and Ogilvie, B. M. R. (1980): Changing proteins on the surface of a parasitic nematode. Nature, 287, 538–541.
10) Philipp, M., Taylor, P. M., Parkhouse, R. M. E. and Ogilvie, B. M. (1981): Immune response to stage-specific surface antigens of the parasitic nematode *Trichinella spiralis*. J. Exp. Med., 154, 210–215.
11) Takahashi, Y., Mizuno, N., Uno, T., Aisaka, A. and Araki, T. (1990a): A spectrum of antibody response with time after *Trichinella spiralis* infection in rats. j. Parasitol., 76, 230–239.
12) Takahashi, Y., Mizuno, N., Uno, T., Tokuda, C. and Araki, T. (1990b): Direct evidence that the cuticle surface of *Trichinella spiralis* muscle larvae shares antigenicity with stichocyte α-granules and the esophagus occupying substance. J. Parasitol., 76, 290–293.