Cross Reactivity of Serum Antibodies from Chickens Immunized with Three Eimerian Species

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ABSTRACT. Cross reactivity of serum antibodies from chicken immunized with *E. tenella*, *E. necatrix* and *E. acervulina* was observed. The enzyme linked immunosorbent assay (ELISA) using soluble antigen prepared from oocysts of each species showed the cross reactivity among chicken serum immunized with the three species of protozoa, especially between *E. tenella* and *E. necatrix*. In immunoblot analysis with *E. tenella* oocyst antigen, 7 major bands were detected by homologous *E. tenella* antiserum and 10 and 6 bands were reacted by *E. necatrix* and *E. acervulina* antiserum respectively. In these antigens, the band of 33 kDa was regarded as cross reactive agent between *E. tenella* and *E. necatrix* because of high reactivity to both of the antiserum. In addition, the 130 kDa antigen that reacted with every antiserum was regarded as common antigen among these three species.—**KEY WORDS:** antibody response, antigen analysis, chicken *Eimeria*.

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Protective immunity to *Eimeria* infection is highly species specific [11]. Serum antibodies, however, are cross reactive to other eimerian species. Danforth and Augustine [2] showed the cross reactivity among various chicken coccidia and turkey coccidia by indirect immunofluorescent antibody technique (IFA). Rose and Mockett [12] also showed the cross reactivity among chicken coccidia and between chicken coccidia and rat coccidia by ELISA. Recently, characterization of antigen from *Eimeria* spp. is thought to be important for the vaccine development. There were some reports with antigen analysis about eimerian species and stages [3, 5–7, 9]. Moreover, species cross reactive antigen were also demonstrated about sporozoites [16] and merozoites [17].

In the present study, we investigated the cross reactivity among three species of chicken *Eimeria* and attempted to demonstrate the species specific and cross reactive antigen of *E. tenella* sporulated oocysts to find the vaccine candidate.

Strain of *E. tenella*, *E. necatrix* and *E. acervulina* obtained from National Institute of Animal Health, Japan, were used for all the experiment. Single comb white leghorn chickens derived from SPF poultry farm of Aburahi Laboratories, Shionogi Pharmaceutical & Co., Ltd. were first immunized at 14 days of age by oral inoculation of 5×10^4 sporulated oocysts. Chickens were inoculated with oocysts of the same species of *Eimeria* as used in the first immunization 14 days after primary inoculation. At the second immunization, inoculation dose were 5×10^4 of *E. tenella*, 5×10^4 of *E. necatrix* and 3×10^5 of *E. acervulina*. Sera were collected 11 days after secondary inoculation and stored at -20°C after inactivation. Soluble antigens of *E. tenella*, *E. necatrix* and *E. acervulina* were prepared from sporulated oocysts respectively. Oocysts purified by the methods of Wagenbach [15] were suspended in sonication buffer (0.1 M Tris-HCl pH 6.8 containing 1 mM phenyl methane sulfonyl fluoride and 200 KU/ml aprotinin) and sonicated 10 times at 25 W for 30 sec. The homogenate was mixed with 0.5 ml of extraction buffer (sonication buffer added with 3% nonidet P-40 and 10 mM ethylene diamine tetraacetic acid) and extracted by standing at 4°C for overnight. The

supernatant was collected as the soluble oocyst antigen and stored at -80°C until use. ELISA was performed according to the method of Voller *et al.* [14] with slight modifications. All of the antigens were coated to 96-well microtitre plate and reacted with every antiserum. The reaction was developed with 2,2'-azino-di[3-ethyl-benzthiazoline sulfate (6)] (ABTS) peroxidase substrate and the absorbances at 405 nm were measured. *E. tenella* antigen was electrophoretically separated by the method of Laemmli [8] and transferred to the nitrocellulose membrane [13]. Separated polypeptides were reacted with the antiserum and detected by Avidin Biotin Complex (ABC) method according to the manufacturer's manual (Vector Laboratories).

Reactivity of serum from chickens immunized with *E. tenella*, *E. necatrix*, or *E. acervulina* was investigated by ELISA using *E. tenella*, *E. necatrix* and *E. acervulina* oocyst antigen.

Figure 1A shows absorbances against *E. tenella* antigen. Homologous *E. tenella* antiserum showed highest absorbances at every dilution. Antiserum to *E. necatrix* showed high absorbances and *E. acervulina* showed low absorbances. Against *E. necatrix* antigen (Fig. 1B), homologous antiserum to *E. necatrix* and antiserum to *E. tenella* showed high absorbances. The absorbances of *E. acervulina* antiserum were also low as that to *E. tenella* antigen. Although every antiserum positively reacted to *E. acervulina* antigen, these levels were lower than that to another two antigens except for the values of homologous *E. acervulina* antiserum (Fig. 1C).

From the results of ELISA, under the immunization procedure we used, *E. tenella* evoked the highest response of serum antibody. In addition, *E. necatrix* also evoked moderately high response, response to *E. acervulina* was low.

Immunoblot analysis demonstrated some of species specific and cross reactive antigens of *E. tenella* sporulated oocysts. Homologous *E. tenella* antiserum strongly reacted seven (33, 59, 93, 106, 109, 113, 130 kDa) polypeptide bands (Fig. 2, Lane 1). *E. necatrix* antiserum proved some of minor bands (37–59, 93, 109 kDa) and major 33 and 130 kDa (Fig. 2, Lane 2). *E. acervulina*

Fig. 1. Reactivity of *E. tenella* (○), *E. necatrix* (□), *E. acervulina* (△) antiserum to *E. tenella* (A), *E. necatrix* (B), and *E. acervulina* (C) oocyst antigen. ●: nonimmunized chicken serum.

antiserum showed 37, 40, 43, 46, 52 and 130 kDa (Fig. 2, Lane 3).

Wisher [16] reported the common antigen among *E. tenella*, *E. acervulina* and *E. maxima* by immunoprecipitation technique with radiolabelled sporozoites. He demonstrated the 43 and 104 kDa antigens specific for *E. tenella* and the 47 kDa common antigen. Xie *et al.* [17] also demonstrated cross reactive merozoites antigen among *E. tenella*, *E. necatrix* and *E. acervulina*. In the present study, species specific and cross reactive antigen of *E. tenella* sporulated oocyst were demonstrated by immunoblotting. Every antiserum strongly reacted to 130 kDa band. It shows that this polypeptide is the one of the prominent and common antigen in these eimerian species. Whereas *E. tenella* and *E. necatrix* antiserum strongly reacted to 33 kDa polypeptide, *E. acervulina* did not recognize. This result may explain the low cross reactivity of *E. acervulina*.

Although only sexual stages of *E. necatrix* develop in the cecum on an ordinary condition, the protozoa can complete their endogenous cycle there like *E. tenella* when the sporozoites of the protozoa are directly injected into it [4]. In addition, it was reported that protective immunity to *E. tenella* was established by direct inoculation of *E. necatrix* sporozoites into the cecum [10]. These

Fig. 2. Immunoblot profiles of *E. tenella* oocyst antigen probed with *E. tenella* (Lane 1), *E. necatrix* (Lane 2) and *E. acervulina* (Lane 3) antiserum.

reports suggest biological and immunological similarity between *E. tenella* and *E. necatrix*. It is well known that these two are only the species among chicken *Eimeria* which parasitize the deep area of lamina propria with high may establish protective immunity against these most pathogenic two species.

Crane *et al.* [1] reported partial cross protection against *E. tenella*, *E. acervulina*, *E. maxima* and *E. necatrix* by immunization with single recombinant antigen from *E. tenella*. In this study, pathogenicity. Cross reactive 33 kDa antigen in this study we observed that *E. tenella* had high antigenicity and the antiserum to this species had cross reactivity to *E. necatrix* and *E. acervulina*. Furthermore, we detected the 130 kDa polypeptide that is commonly recognized by antiserum to these *Eimeria*. This 130 kDa polypeptide can be one of the candidate for multivalent vaccine.

REFERENCES

1. Crane, M. S. J., Goggin, B., Pellegrino, R. M., Ravino, O. J., Lange, C., Karkhanis, Y. D., Kirk, K. E., and Chakraborty, P. R. 1991. *Infect. Immun.* 59: 1271-1277.
2. Danforth, H. D. and Augustine, P. C. 1983. *Poult. Sci.* 62: 2145-2151.
3. Danforth, H. D. and McAndrew, S. J. 1987. *J. Parasitol.* 73: 985-992.
4. Horton-Smith, C. and Long, P. L. 1965. *Parasitology* 55: 401-405.
5. Jenkins, M. C. and Dame, J. B. 1987. *Mol. Biochem. Parasitol.* 25: 155-164.

6. Kawazoe, U., Tomley, F. M., and Frazier, J. A. 1992. *Parasitology* 104: 1-9.
7. Karkhanis, Y. D., Nollstadt, K. A., Bhogal, B. S., Ravino, O., Pellegrino, R., Crane, M. S., Murray, P. K., and Turner, M. J. 1991. *Infect. Immun.* 59: 983-989.
8. Laemmli, U. K. 1970. *Nature (Lond.)* 227: 680-685.
9. Reduker, D. W. and Speer, C. A. 1986. *J. Parasitol.* 72: 901-907.
10. Rose, M.E. 1967. *Parasitology* 57: 567-583.
11. Rose, M. E. 1982. Host immune response. pp. 329-359. In: *The Biology of the Coccidia* (Long, P. L. ed.), Univ. Park Press, London.
12. Rose, M. E. and Mockett, A. P. A. 1983. *Parasite Immunol.* 5: 479-490.
13. Towbin, H. T., Stachelin, T., and Gordon, J. 1979. *Proc. Natl. Acad. Sci. U.S.A.* 76: 4350-4354.
14. Voller, A., Bidwell, D. E., and Bartlett, A. 1976. *Bull. WHO.* 53: 55-65.
15. Wagenbach, G. E., Challey, J. R., and Burns, W. C. 1966. *J. Parasitol.* 52: 1222.
16. Wisher, M. H. 1986. *Mol. Biochem. Parasitol.* 21: 7-15.
17. Xie, M., Gilbert, J. M., and McDougald, L. R. 1992. *J. Parasitol.* 78: 82-86.