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Research Note

Protein Composition and Antigenicity of the Inner Membrane of *Toxoplasma gondii* Treated with Hemolysin from *Pseudomonas aeruginosa*

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Ultrastructural observations of motile stages of coccidia including *Toxoplasma gondii* show that they are surrounded by a pellicle comprising a typical plasmalemma and two layers of an inner membrane which is formed by a system of flattened vesicles (Vivier and Petitprez, 1969; Dubremetz, 1975). Although surface antigens of the outer membrane of *T. gondii* have been extensively studied (Couvreur *et al.*, 1988; Handman *et al.*, 1980), little is known about the components and antigenicity of the inner membrane. We previously found that treatment of *Toxoplasma* tachyzoites with a high concentration of the hemolysin from *Pseudomonas aeruginosa* yielded a membrane-rich fraction free from all intracellular components except some conoids and microtubules. The membrane in our preparation differed in its morphology from the outer membrane so that it was considered as the inner membrane of the tachyzoites (Makioka *et al.*, 1986). This paper describes the protein composition of the inner membrane obtained by hemolysin treatment and also reactivity of its major protein with antisera from mouse, rabbit and human infected with *T. gondii.*

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The inner membrane-rich fraction was prepared as previously described (Makioka *et al.*, 1986; Tryon, 1979). Briefly, *Toxoplasma* tachyzoites of the RH strain were obtained from 2-day-infected female outbred ddY mice. They were purified with 8 units (1 unit = amount of hemolysin needed to lyse 1 ml of 1% red blood cells) of hemolysin to remove host cells and then treated with 80 units of hemolysin in the presence of DNase and RNase (both at a concentration of 1 mg/ml). For electron microscopy, samples were fixed in 2.5% glutaraldehyde and 2% OsO_4, dehydrated in acetone and embedded in Epon. Thin sections were cut on an Ultracut microtome, post-stained with uranyl acetate and lead citrate and viewed on a JEM-100s electron microscope (Makioka *et al.*, 1986).

Tryon (1979) reported that treatment of *Toxoplasma* tachyzoites with high concentration of hemolysin from *Pseudomonas aeruginosa* left the outer membrane intact. However, we found that the membrane remaining after treatment with the hemolysin was not the outer but the inner membrane of the tachyzoites (Makioka *et al.*, 1986). The action of *Pseudomonas* hemolysin is considered to be detergent-like (Hirayama and Kato, 1982). Similar resistance of the inner membrane to Triton X-100 was observed (Cintra and De Souza, 1985). An electron micrograph of the inner membrane in our preparation is shown in Fig. 1. This preparation did not contain the outer

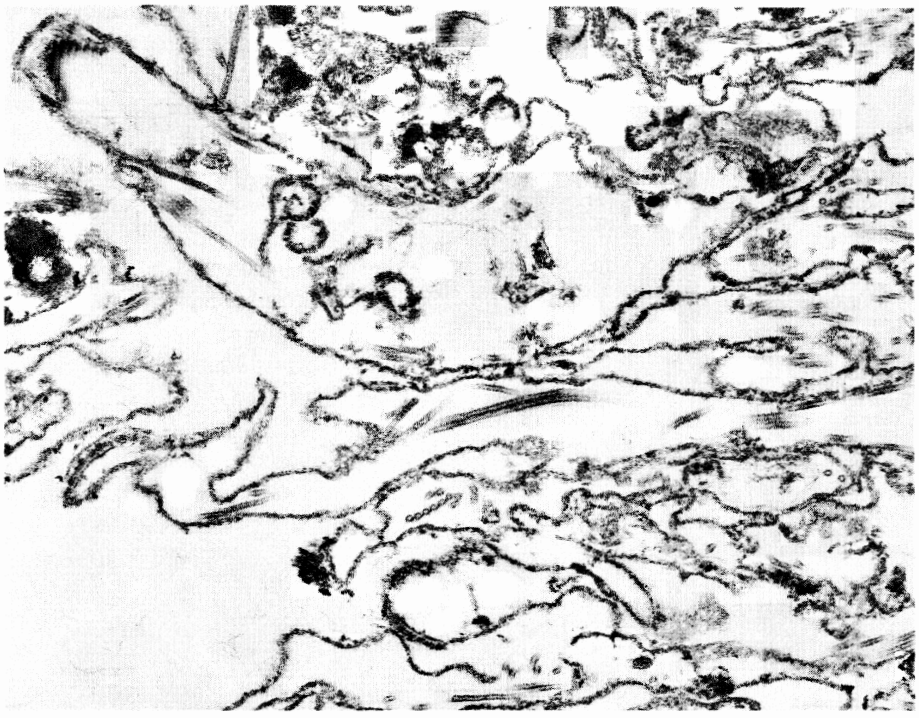

Fig. 1 Membrane-rich fraction after treatment of *T. gondii* tachyzoites with *Pseudomonas* hemolysin, which is almost exclusively composed of the inner membrane. (×45,000)

membrane but was contaminated with some intracellular components including conoids and microtubules.

Proteins present in our inner membrane-rich fraction were analysed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). This was performed using a Tris-glycine buffer, pH 8.3, containing 0.1% SDS as described by Laemmli (1970) on 15% polyacrylamide slab gels. Gels were stained with Coommasie blue. The results showed that it contained a 42-kDa protein (P42) as a major one and several minor proteins (Fig. 2). Nonreducing conditions for SDS-PAGE did not change mobility of the protein (data not shown), suggesting that it had a monomeric form. Recently, Foussard *et al.* (1990) isolated the pellicle of *Toxoplasma* tachyzoites by a discontinuous sucrose gradient and magnetic microspheres and analysed its protein composition. They showed that the pellicle contained five major proteins (mol. wt. 19, 22, 35, 43 and 79 kDa). It remains unclear whether the P43 in their study corresponds to our P42 because their pellicle preparation contained both the outer and inner membranes. Schwartzman *et al.* (1985) showed that monoclonal antibodies specific for mammalian β-tubulin recognized a major protein of 53–54 kDa in *T. gondii,* consistent with β-tubulin. Although our preparation of the inner membrane was contaminated by microtubules, the 53–54-kDa protein appeared as a minor band on SDS-PAGE.

We next examined antigenicity of the P42 by immunoblotting. Transfer of separated antigens on SDS-PAGE to a nitrocellulose membrane was carried out in 25 mM Tris-HCl buffer, pH 8.3, contained 192 mM glycine and 20% (v/v) methanol, with a commercial blotting apparatus (ATTO Co., Tokyo). The immunoblotting was performed as described by Partanen *et al.* (1983). Briefly, the nitrocellulose membrane was soaked in 0.01M phosphate-buffered saline, containing 10% fetal bovine serum and 0.2% (v/v) Triton X-100 and then incubated with 1:100 diluted

antisera from mouse, rabbit and human infected with *T. gondii*. Three ddY mice and two rabbits were each infected with cysts of the Beverley strain of *T. gondii* and 2 months later antisera were obtained and pooled. Human antiserum was obtained at Jikei University Hospital, Tokyo. All antisera showed a dye test titer of 1:1024. After washing, the membrane was incubated with peroxidase-conjugated goat anti-mouse, rabbit or human IgG (Cappel laboratories, PA). After washing, the membrane was developed with 3, 3-diaminobenzidine HCl and H_2O_2. The results showed that the minor band just below the P42 strongly reacted with those antisera but the P42 reacted very weakly (Fig. 3), suggesting that the antigenicity of the P42 was weak. It remains unclear whether the minor protein which strongly reacts with those antisera is a minor component of the inner membrane because our preparation is contaminated with some intracellular components.

Biochemical and immunological investigations of the inner membrane of *T. gondii* have been

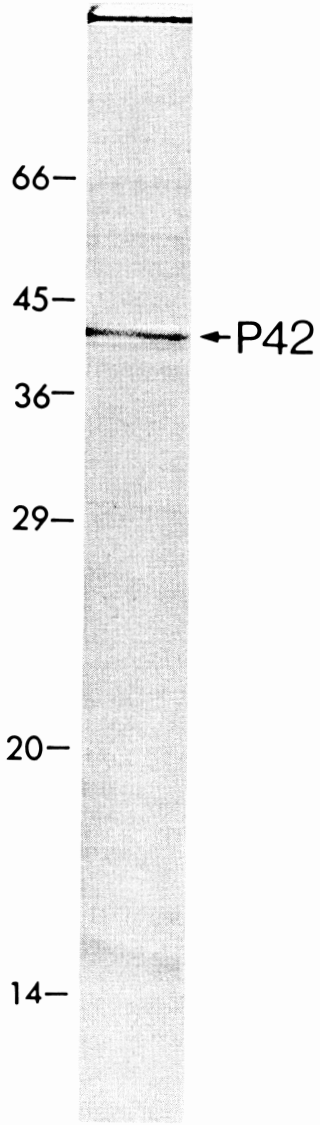

Fig. 2 SDS-PAGE of the inner membrane-rich fraction. Arrow indicates a major protein of 42 kDa (P42). Numbers on the left are molecular-weight markers (kDa).

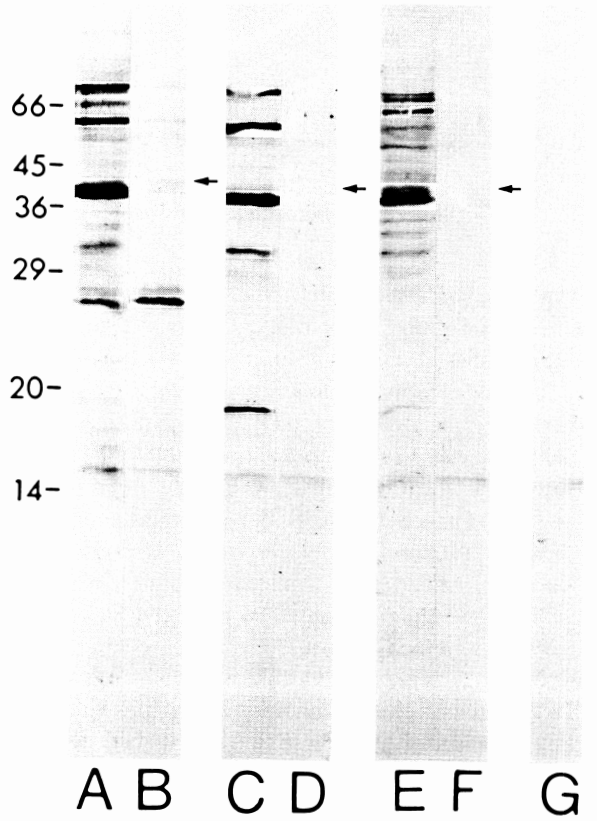

Fig. 3 Reactivity of the P42 with antisera from mouse, rabbit or human infected with *T. gondii*. (A) Rabbit antiserum, (B) Normal rabbit serum, (C) Mouse antiserum, (D) Normal mouse serum, (E) Human antiserum, (F) Normal human serum, and (G) No serum. Arrows indicate position of P42.

few. Our present study, for the first time, identified its major protein and may contribute to its further characterization.

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References

1) Cintra, W. M. and De Souza, W. (1985): Immunocytochemical localization of cytoskeletal proteins and electron microscopy of detergent extracted tachyzoites of *Toxoplasma gondii*. J. Submicrosc. Cytol., 17, 503–508.
2) Couvreur, G., Sadak, A., Fontier, B., Dubremetz, J. F. (1988): Surface antigens of *Toxoplasma gondii*. Parasitology, 97, 1–10.
3) Dubremetz, J. F. (1975): La genese des mérozoites chez la coccidie *Eimeria necatrix*. Etude ultrastructurale. J. Protozool., 22, 71–84.
4) Foussard, F., Gallois, Y., Tronchin, G., Robert, R. and Mauras, G. (1990): Isolation of the pellicle of *Toxoplasma gondii* (Protozoa, Coccidia): characterization by electron microscopy and protein composition. Parasitol. Res., 76, 563–565.
5) Handman, E., Goding, J. W. and Remington, J. S. (1980): Detection and characterization of membrane antigens of *Toxoplasma gondii*. J. Immunol., 124, 2578–2583.
6) Hirayama, T. and Kato, I. (1982): Novel methyl rhamnolipids from *Pseudomonas aeruginosa*. FEBS Lett., 139, 81–85.
7) Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680–685.
8) Makioka, A., Kobayashi, A., Matsuura, M. and Homma, J. Y. (1986): An electron microscopic study on *Toxoplasma gondii* treated with heat-stable hemolysin from *Pseudomonas aeruginosa*. Jpn. J. Parasitol., 35, 149–151.
9) Partanen, P., Turunen, H. J., Paasivuo, R., Forsblom, E., Suni, J. and Leinikki, O. (1983): Identification of antigenic components of *Toxoplasma gondii* by an immunoblotting technique. FEBS Lett., 158, 252–254.
10) Schwartzman, J. D., Krug, E. C., Binder, L. I. and Payne, M. R. (1985): Detection of the microtubule cytoskeleton of the coccidian *Toxoplasma gondii* and the hemoflagellate *Leishmania donovani* by monoclonal antibodies specific for β-tubulin. J. Protozool., 32, 747–749.
11) Tryon, J. C. (1979): *Toxoplasma gondii*: ultrastructure and antigenicity of purified tachyzoite pellicle. Exp. Parasitol., 48, 198–205.
12) Vivier, E. and Petitprez, A. (1969): Le complexe membranaire superficiel et son évolution lors de l'élaboration des individus-fils chez *Toxoplasma gondii*. J. Cell Biol., 43, 329–342.