[Jpn. J. Parasitol., Vol. 35, No. 2, 149–151, April, 1986]

Research Note

An Electron Microscopic Study on *Toxoplasma gondii* Treated with Heat-Stable Hemolysin from *Pseudomonas aeruginosa*

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(Received for publication; November 26, 1985)

Key words: *Toxoplasma gondii*, Hemolysin, *Pseudomonas aeruginosa*, Ultrastructure

The heat-stable hemolysin is a glycolipid produced by some strains of *Pseudomonas aeruginosa* (Liu, 1957). Tryon *et al.* (1978) have noted that the hemolysin lyses white blood cells and animal tissue cells in culture, as well as red blood cells of a number of species. They have also demonstrated that while host cells are lysed by this glycolipid, *Toxoplasma gondii* tachyzoites remain intact. They advocated that this technique is useful for obtaining pure and viable tachyzoites at a high yield. Furthermore, Tryon (1979) demonstrated that when the purified tachyzoites were treated with higher concentration of hemolysin, the organisms yielded the outer pellicular membrane with two subcellular components (conoid and microtubules), the latter two being solubilized by a further treatment with protease.

We took interest in this report since no successful isolation of the outer membrane of *Toxoplasma* had been made. We tried to isolate the heat-stable hemolysin from *Pseudomonas aeruginosa*. The hemolysin samples thus obtained were applied to *Toxoplasma* tachyzoites and results of isolation trials of the parasite membrane were examined by electron microscope.

Eleven strains of *P. aeruginosa* were screened for hemolysin production. Culture conditions were essentially the same as those described previously (Larson and Hart, 1975; Johnson and Boese-Marrazzo, 1980). In brief, those strains were cultured in 60 ml of a peptone medium (containing 1% each of peptone, glycerol and NaCl at pH 7.0) in a cotton-stoppered 500-ml Erlenmeyer flask at 30°C on a shaker for 2 or 6 days. After centrifugation at 12,000 x *g* for 15 min, the culture supernatants were autoclaved at 115°C for 10 min before hemolysin assay. Hemolysin in the culture supernatant was titrated by two-fold dilution in phosphate-buffered saline, pH 7.2 (PBS) as follows. To 0.5 ml of diluted hemolysin was added 0.5 ml of 1% rabbit erythrocytes. The cell suspensions were incubated for 2 hr at 37°C, and the highest dilution showing complete hemolysis was taken as endpoint with which the reciprocal titer was determined. As shown in Table 1, two of those strains produced no detectable hemolysin activity, and the other 9 yielded titers ranging from 2 to 16. The M2 strain yielding the highest titer was selected for large-scale preparation of hemolysin. For this, 300-ml cultures were grown in 2,000-ml Erlenmeyer flasks for

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Table 1 Hemolysin production by various strains of *Pseudomonas aeruginosa*

P. aeruginosa strains	Hemolytic titer* 2d	6d
Scharmann	< 2	4
M2	4	16
Mc5	< 2	< 2
No. 5	2	8
P1	< 2	8
P2	< 2	4
P3	2	8
P4	< 2	2
P4-103	< 2	< 2
P5	< 2	4
P9	< 2	2

*Hemolytic titers were measured on the autoclaved culture supernatants from 2 or 6 days of cultures.

5 or 6 days at 37°C in the peptone medium. Hemolysin was purified as described by the previous studies (Larson and Hart, 1975; Johnson and Boese-Marrazzo, 1980). In brief, hemolysin in the culture supernatant was adsorbed onto charcoal, followed by successive elution with ethanol. The ethanol eluate was then concentrated and dialyzed overnight against distilled water. After precipitation with 5 M NaCl, hemolysin was collected by centrifugation and dissolved in PBS.

Toxoplasma tachyzoites of the RH strain were obtained from 2-day-infected 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 and then treated with 80 units of hemolysin at the presence of DNase and RNase (both at a concentration of 1 mg/ml) as described by Tryon (1979). For electron microscopy, all 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. Figure 1 shows the preparation obtained by treatment of *Toxoplasma* tachyzoites with hemolysin. Conoid and microtubules remained intact as described by Tryon (1979). However, the membrane

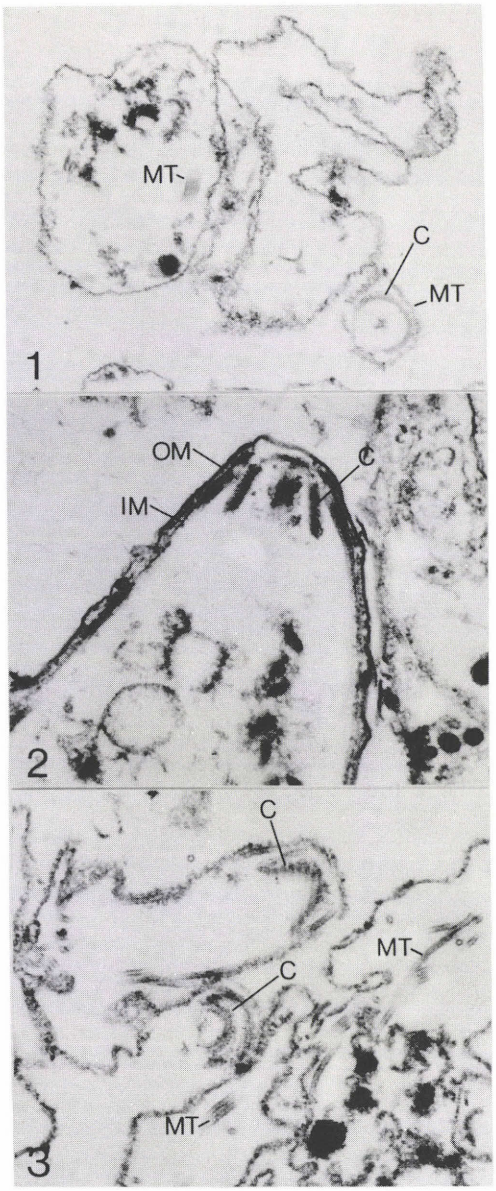

Fig. 1 *Toxoplasma* tachyzoites treated with hemolysin. Conoid (C), microtubules (MT). (× 39,700)

Fig. 2 *Toxoplasma* ghosts obtained by lysing the tachyzoites in distilled water. Outer membrane (OM), inner membrane (IM). (× 63,500)

Fig. 3 Hemolysin-treated *Toxoplasma* ghosts. (× 63,500)

obtained in this experiment differed entirely from the outer membrane which was considered morphologically analogous to a typical unit membrane. The membrane in our preparation did not have smooth surface and looked fluffy so that it was considered as the inner membrane of the tachyzoites. To confirm this further, we prepared ghosts by lysing the tachyzoites overnight in distilled water. They were then examined directly or after treated with hemolysin under an electron microscope. Both the outer and inner membranes remained in the control ghosts and were distinguishable one from the other (Fig. 2). In contrast, hemolysin-treated ghosts yielded uniform membrane which exhibited similar configuration to that of the inner membrane in the untreated ghosts (Fig. 3). Membranes with the unit structure were not found in the preparation.

From these observations, the tachyzoites membrane obtained by the hemolysin treatment was considered to be the inner membrane. Reason for the discrepancy between the results by the previous study (Tryon, 1979) and our present study is not clear.

Acknowledgements

We wish to thank Dr. M. Yamaguchi, Jikei University School of Medicine, for electron microscopy and also to Dr. T. Endo, National Institute of Health of Japan, for his valuable advice.

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短　報

緑膿菌ヘモリジンで処理したトキソプラズマ増殖型の電子顕微鏡的観察

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トキソプラズマ(T_P) 増殖型虫体の外膜の分離に緑膿菌由来の耐熱性ヘモリジンが有用であるとする報告がなされている．これを確認するため，緑膿菌ヘモリジンを調製し，原報に従い，T_P 増殖型虫体に作用させ，電顕で観察し，既報と異なる成績を得たので報告する．ヘモリジン処理により得られた膜は，外膜とは異なり，内膜と思われた．そこでさらにこれを確認するため，T_P 虫体を蒸留水中で破裂させてゴーストを得，これをさらにヘモリジンで処理し，無処理のものと比較した．無処理の T_P ゴーストには外膜，内膜とも残存し，それらは明瞭に識別できた．一方，ヘモリジン処理群では外膜に相当する構造は観察されず，すべて内膜と形状が同じであった．