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The Membrane Characters of Different Forms of *Trypanosoma cruzi* (II)

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The differences of coating antigen on the surface membrane among amastigote form (A form) in the monophasic medium, cultured epimastigote form (E from) and trypomastigote form (T form) in the blood of infected mouse of *Trypanosoma cruzi* (*T. cruzi*) were demonstrated by employing the fluorescent antibody technics (Kanbara *et al.* 1974). Alves and Colli (1974) suggested differences in membrane structure between blood forms and cultured epimastigote of *T. cruzi* through agglutination assay with concanavalin A.

Electronmicroscopically, existence of surface coating structures on the plasma membrane of T and A forms of *T. cruzi* was shown by Sanabria and Aristimuno (1969, 1970, 1972), Inoki *et al.* (1972) and Kanbara *et al.* (1974). Vickerman (1969 a, b) showed that the surface coat overlies the plasma membrane in blood stream form of salivarian trypanosomes but not in culture/vector form.

In the present experiment, the further observation on antigenicity of coating substances on A and T forms of *T. cruzi* derived from various conditions was performed by the fluorescent antibody staining. The relationship between surface coating antigen and infectivity (or virulence) of *T. cruzi* was discussed.

Materials and Methods

Preparation of parasites

Tulahuen strain of *T. cruzi* obtained from the National Institute of Health, Bethesda, Maryland, U.S.A. through the Keio University, Tokyo, Japan and maintained in mice by syringe passage and in the modified NNN diphasic medium in our laboratory was used. In the present experiment, T and A forms of *T. cruzi* were harvested from each of the following system.

(1) Parasite from infected mice

Amastigote (A-M) in peritoneal fluid and trypomastigote (T-M) in blood were obtained from heavily infected mice and used for infection and immunofluorescent study.

(2) Parasites taken from infected mouse blood and transferred to cell culture system

The blood from mice infected with blood forms one week earlier was withdrawn in Alsever's solution and centrifuged at 1,000 r.p.m. for 10 minutes. The supernatent was recentrifuged at 3000 r.p.m. for 10 minutes. The collected cells were dispersed in cell culture medium consisting of 5 parts of Eagle MEM and 1 part of calf serum. Five million of blood forms of the parasite were transferred into established fibroblast cell culture from Balb-C mouse in a culture tube (4×10 cm) and maintained at 37°C. Medium changing was done every 4 to 7 days. A (A-CB) and T (T-CB) forms released from destructed fibroblast cells were collected 70 days after inoculation and used for the experimental work.

(3) A and T forms in cell culture originated from cultured E froms (A-CE and T-CE)

Around 10^8 E forms kept in the diphasic medium were transferred into fibroblast cell culture, and A and T forms produced after five months were used.

Inoculation to the animals

Four to 6 weeks-old dd-O mice were inoculated with 2×10^4, 10^5 and 5×10^5 blood forms (T-M), 5×10^5 T-CB, 5×10^6 T-CE and 10^7 cultured E forms. Parasitemia was examined through observation of trypanosomes in a drop of tail blood at 7 and 14 days after inoculation.

Fluorescent antibody staining of the parasite

The fluorescent antibody to the cultured A forms in the monophasic medium prepared in the previous experiment (Kanbara *et al.* 1974) was used. The used fluorescent antibody had 1.1 of F/P ratio and 3.9 mg/ml of protein. Absorbtion with E forms and staining were performed by the same methods as shown in the correspondence.

Results

Table 1 shows the time course of parasitemia and the death rate of mice inoculated with respective cells. T-CB showed as strong virulence to mice as the blood form (T-M) but neither T-CE nor cultured E form. 10^5 and 5×10^5 T-M and 5×10^5 T-CB invariably resulted in a fetal infection in mice within 20 days after inoculation. Infected mice with both 10^7 cultured E forms and 5×10^6 T-CE survived without showing any symptom. Four of 5 mice injected with T-CE showed very low parasitemia 7 days after inoculation but it disappeared 14 days. Titers of the absorbed and unabsorbed fluorescent antibody to respective froms are shown on Table 2.

All the studied A forms gave ring form fluorescence pattern and same titer when they were reacted with the absorbed and unabsorbed fluorescent-antibody (Photos. 1, 2,3). T-CE, on the other hand, took lower titer and the speckled fluoresence pattern and agglutinated (Photo. 4). Most of the T-CB and T-M parasite, however, were not stained by either absorbed or unabsorbed fluorescent antibody. Thus the difference in the affinity to the antibody conjugate between the infective (T-CB, T-M) and the non-infective (T-CE) trypomastigote was observed so far as present immunofluorescent study concerned.

Discussion

The cultured E form employed in the present experiment has been maintained more than two years in the modified NNN diphasic medium and is deficient in infectivity to mouse. This strain did not show any virulence even after the development of A and T forms in the cell culture. On the other hand, T form produced in cell culture 70 days after inoculation of blood forms showed as strong infectivity as T forms

Table 1 Infectious course of mice inoculated with trypanosomes from various condition

Dose of flagellates inoculated	No. of used mice	No. of infected mice at 7 days	No. of infected mice at 14 days	No. of dead mice by 14 days	No. of dead mice from 15 to 20 days	No. of survived mice more than 3 months
T-M 2×10^4	5	0	5	0	3	0
T-M 10^5	5	5	—	0	5	0
T-M 5×10^5	10	10	—	8	2	0
T-CB 5×10^5	5	5	—	5	—	0
T-CE 5×10^6	5	4	0	0	0	5
E form 10^7	5	0	0	0	0	5

T-M: trypomastigote from infected mouse blood. T-CB: trypomastigote produced in fibroblast cell culture 70 days after inoulation of blood forms. T-CE: trypomastigote produced in fibroblast cell culture 5 months after inoculation of cultured epimastigotes.
E forms: epimastigote maintained in modified NNN medium.

Table 2 Titers of absorbed and unabsorbed fluorescent antibody with cultured E forms to respective forms from various condition

	Fluorescence titer						
	E form	A form from monophasic medium	A-CB	A-CE	T-M	T-CB	T-CE
Unabsorbed	1 : 16	1 : 32	1 : 32	1 : 32	none	none	1 : 8
Absorbed	none	1 : 16	1 : 16	1 : 16	none	none	1 : 4

A-CB: amastigote multiplied in fibroblast cell culture 70 days after inoculation of blood forms

A-CE: amastigote multiplied in fibroblast cell culture 5 months after inoculation of cultured epimastigotes

in blood of infected mouse. A forms obtained under various conditions consistently showed ring pattern when they were stained by the fluorescent antibody which was absorbed with cultured E forms. It suggested that all the examined A forms have the same antigenic coating substances on the plasma membrane or at least share one common antigen. This sort of coating antigen was found persisting partially on T forms from cell culture (T-CE) after transfer of E form, but not on T forms from infected mouse blood (T-M) and produced in cell culture (T-CB) 70 days after inoculation with blood forms.

Hence, the facts shown above will reason the speculation that the variation of the coating antigen occurs in virulent forms in infected mouse and also in cell culture accompanied by A-T form change. Avirulent forms, on the other hand, are unable to complete such antigenic variation due to the form change. Alves and Colli (1974) noted that virulence might be associated, at least in part, with the surface properties of the cells at their various stages of differentiation, as suggested by their different behavior in the presence of the lectin, while the effect to amastigote and avirulent trypomastigote by concanavalin A remains unknown. Regarding the variation of surface antigen, the difference between virulent and avirulent trypomastigote observed in the present immunofluorescent study arises interest even though it may be still controversial that antigenic variation of coating substance plays important role in infectivity of *T. cruzi*. Further characterization of the surface antigen is required.

Summary

The surface antigen of amastigote and trypomastigote forms of virulent and nonvirulent system of *Trypanosoma cruzi* has been compared by immunofluorescent method. Respective forms were obtained in (1) the parasite harvested from peritoneal exudate and blood of the infected mouse (virulent) (2) the parasite originally obtained from blood of infected mouse, then maintained in cultured fibroblast cell for 70 days (virulent) (3) the parasite originated from epimastigote in the diphasic medium, then kept in tissue culture for 5 months (avirulent). On application of FITC labelled anti-amastigote globulin, both forms of the avirulent parasite were stained, while in the case of virulent system, only A form resulted in a positive fluorescence pattern. T form from the same series, however, did not react with the conjugate. These results seem to reflect that the variation of the surface antigen is associated with the virulence of the *Trypanosoma cruzi*.

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Trypanosoma cruzi の膜表面構造に関する研究 (II)

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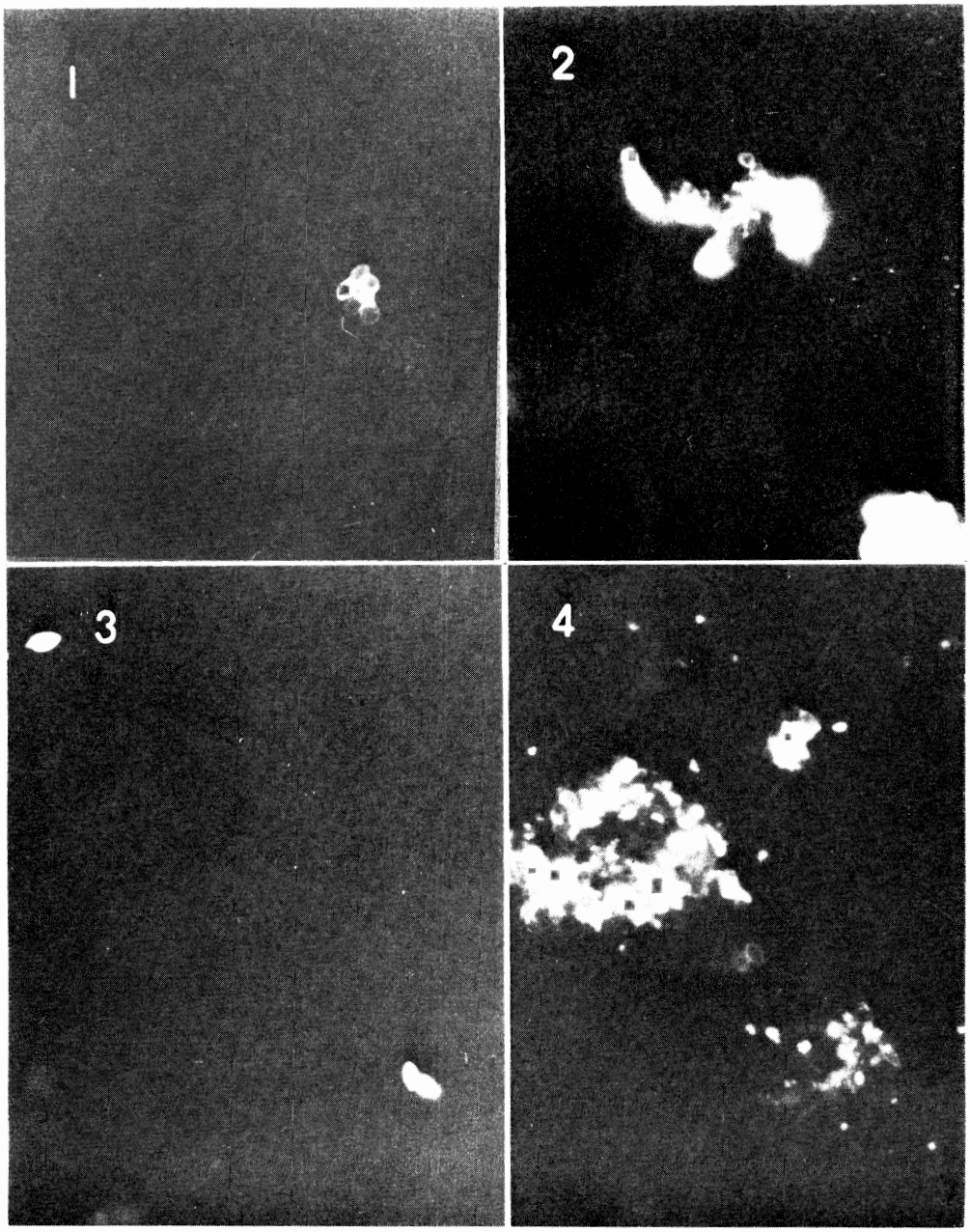
前回の実験に引続いて，いろいろな条件下に増殖，発達してくる *Trypanosoma cruzi* の Trypomastigote, Amastigote について，表面抗原を螢光抗体法により観察した。その結果マウス腹水中，マウス線維芽細胞培養中に出現する Amastigote も，感染マウスより移植後，液体培養基中に出現する Amastigote と同じものか，少くとも共通抗原性のある物質によって被れている事が示された。更に病原性の強い Trypomastigote は Amastigote から Trypomastigote への形態変化に伴い表面抗原を変化させるが，病原性の弱いものでは，この抗原変化が完全に行われず，Amastigote と同様な抗原物質が部分的に残っている事が判明した。



Photos 1–4. Staining of trypanosomes by fluorescent antibody subjected to absorption with cultured epimastigotes.

Photo 1. Amastigote appeared in fibroblast cell culture (A-CB) 70 days after inoculation of blood forms.

Photo 2. Amastigote appeared in fibroblast cell culture (A-CE) 5 months after inoculation of epimastigote.

Photo 3. Amastigote appeared in peritoneal exudate of infected mouse (A-M) All amastigotes examined showed ring-form pattern.

Photo 4. Trypomastigotes produced in fibroblast cell culture 5 months after inoculation of epimastigote (T-CE) were agglutinated and stained partially but not as ring-form.