[Jpn. J. Parasitol., Vol. 35, No. 4, 313–321, August, 1986]

Polyclonal B-cell Activation and Immunosuppression Induced by Fraction Derived from the Surface of *Trypanosoma gambiense*

MIKIO OKA

(Received for publication; January 23, 1986)

Key words: *Trypanosoma gambiense,* mouse, polyclonal B-cell activation, immunosuppression

Introduction

Salivarian trypanosomes are commonly characterized by profound polyclonal B-cell activation and immunosuppression (Goodwin *et al.*, 1972; Greenwood, 1974; Murray *et al.*, 1974; Ackerman and Seed, 1976). Whole or fractionated trypanosomal homogenate as well as living parasites has been reported to cause these phenomena (Hudson *et al.*, 1976; Clayton *et al.*, 1979; Oka *et al.*, 1984). Although several workers reported on the properties of trypanosome-derived substance(s) responsible for these immunological alterations (Assoku *et al.*, 1979; Sacks *et al.*, 1982; Diffley, 1983), it has still been controversial which component(s) in the mechanically disrupted cell homogenate is biologically and immunologically active.

The polyclonal B-cell activation was induced by the purified trypanosome surface membrane as well as the endoplasmic reticulum (Sacks *et al.*, 1982). It appears that the mitogenic activity is manifested by a membrane-binding component of the parasites. In preliminary experiments, separation of the mitogenic component from the cell homogenate was not successful using detergents, such as deoxycholate or Nonidet P-40. In this study, separation of the polyclonal B-cell activator and immunosuppressive substance from the plasma membrane was achieved by treating living trypanosomes with trypsin and the partial properties of these components were examined. The data presented here suggests that the polyclonal B-cell activation and immunosuppression appear to be induced by different materials from the surface of parasites.

Department of Parasitology, School of Medicine, The University of Tokushima, Kuramoto-cho 3, Tokushima 770, Japan.

This work was supported by a Grant-in-Aid (No. 59770277) from the Ministry of Education Science and Culture, Japan.

Materials and Methods

Female ddY mice (7–10 weeks old) served as the host animal. They were bred under specific-pathogen free conditions at Tokushima Experimental Animal Laboratory (Tokushima, Japan).

Trypanosoma gambiense (Wellcome strain) was passed through the mice every 3 days. Organisms were separated from whole heparinized blood by the method of Lanham and Godfrey (1970) and then washed twice with phosphate-buffered saline (PBS).

To prepare the subcellular fraction of trypanosomes, parasites were disrupted by repeated freeze-thawing followed by trituration with a Teflon homogenizer at 4°C, yielding a cell homogenate of the parasites. The cell homogenate was centrifuged at 12,000 × *g* for 30 min to remove nuclei, kinetoplasts, and large cell debris (Sacks *et al.*, 1982). The

supernatant was then centrifuged at 144,000 x *g* for 1 hr to prepare the final supernatant and sediment fractions. The living trypanosomes (1×10^9 organisms/ml) were incubated with 50 μg/ml of trypsin in PBS for 10 min at room temperature. The reaction was terminated by the addition of tosyl-L-lysin chloromethylketone. The trypsin-treated parasites were centrifuged at 1,000 x *g* for 10 min, and the supernatant was passed through a Millipore filter (pore size 0.3 μm). The cell homogenate of trypsin-treated parasites was prepared by the above method.

To examine the biochemical properties of the trypanosome fractions, the 144,000 x *g*-sediment fraction was incubated with protease (100 μg/ml), ribonuclease (RNase, 100 μg/ml) and deoxyribonuclease (DNase, 100 μg/ml) in PBS at 37°C for 3 hr. The 1,000 x *g*-supernatant from trypsin-treated trypanosomes was incubated with pronase (100 μg/ml) in PBS at 37°C for 3 hr. For periodate treatment, the 1,000 x *g*-supernatant was incubated with 20 mM sodium metaperiodate in PBS, and the reaction mixture was kept in dark at 20°C for 3 hr. A part of the 1,000 x *g*-supernatant was dialyzed against PBS containing 1 mM tosyl-L-lysin chloromethylketone at 4°C for 3 hr across a membrane with a pore size of approximately 10,000 daltons.

The effect of the trypanosome cell fractions on the background IgM splenic plaque-forming cells (PFC) to sheep red blood cells (SRBC) was assayed 4 days after the intraperitoneal injection of the trypanosome cell fractions using the method of Jerne and Nordin (1963) since an increase in the number of the background PFC to SRBC is often used to demonstrate the polyclonal B-cell activation (Assoku *et al.*, 1979; Diffley, 1983). To study the primary antibody response to SRBC, mice were immunized intraperitoneally with 3×10^7 SRBC 4 days after the injection of the cell fractions, and then IgM PFC response was assayed 5 days after the priming. The difference in the splenic PFC response was analyzed statistically by Student's *t* test. Results were considered significant at the level $P < 0.05$.

For electron microscopic observation, untreated and trypsin-treated trypanosomes were fixed at 4°C for 1 hr in a mixture of 2.5% glutaraldehyde and 4% sucrose in 0.05 M cacodylate buffer (pH 7.3) and postfixed for 2 hr in 1% O_SO_4. The samples were rinsed with distilled water, dehydrated in acetone, and embedded in Epon. Ultrathin sections were stained with uranylacetate and lead citrate, and then examined with a Hitachi HU-12 electron microscope.

To determine the protein composition, the 1,000 x *g*-supernatant from trypsin-treated trypanosomes was analyzed using 15% polyacrylamide gel electrophoresis under reducing conditions in the presence of 0.1% sodium dodecyl sulfate. Gel tracks were stained for proteins with silver staining (Oakley *et al.*, 1980).

The following enzymes and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA): protease (type IV), pronase E, trypsin, RNase, DNase and tosyl-L-lysin chloromethylketone. Sodium metaperiodate was purchased from Wako Pure Chemical Industries (Japan).

Results

For the preliminary study, two *T. gambiense* subcellular fractions, 144,000 x *g*-sediment and -supernatant, were examined for polyclonal B-cell activation. Although an increase of background PFC response to SRBC was induced by injection of the sediment, there was no increase of the background PFC response by stimulation with the supernatant (Table 1). The polyclonal B-cell stimulating activity in the sediment fraction was sensitive to protease and resistant to RNase or DNase (Table 2). The protease-sensitivity of the active component appeared to suggest that it might be a membrane bound protein. It seemed possible that the active component could be separated from the plasma membrane of living trypanosomes by a selective proteolytic enzyme such as

Table 1 Effect of *Trypanosoma gamiense* 144,000 x *g*-supernatant and -sediment fractions on background anti-SRBC PFC

Fraction*	Dose (μg)	Anti-SRBC PFC per spleen† ± SE	Stimulation index‡
PBS	–	40 ± 14	–
Supernatant	1,000	65 ± 17	1.6
Sediment	1,000	1,280 ± 343	32.0
	500	1,265 ± 137	31.6
	50	2,675 ± 591	66.9
	10	785 ± 201	19.6

* Phosphate-buffered saline (PBS) or trypanosome fractions were injected intraperitoneally.

† Four days after administration of test fractions, background IgM plaque-forming cells (PFC) to sheep red blood cells (SRBC) was measured. Values are arithmetic means, five mice per group.

‡ Stimulation index = anti-SRBC PFC per spleen (test fraction)/anti-SRBC PFC per spleen (PBS).

Table 2 Effect of *Trypanosoma gambiense* 144,000 x *g*-sediment fraction after treating with enzymes on background anti-SRBC PFC

Fraction*	Treatment†	Anti-SRBC PFC per spleen‡ ± SE	Stimulation index§
PBS	–	100 ± 20 (A)	–
Sediment	–	1,475 ± 231 (B)	14.8
Sediment	DNase	1,165 ± 396 (C)	11.7
Sediment	RNase	1,985 ± 490 (D)	19.9
Sediment	Protease	440 ± 165 (E)	4.4

* After treatment, phosphate-buffered saline (PBS) or sediment fraction (50 μg) were injected intraperitoneally.

† See materials and methods.

‡ Four days after administration of test fractions, background IgM plaque-forming cells (PFC) to sheep red blood cells (SRBC) was measured. Values are arithmetic means, five mice per group. Difference from (A) was significant in (B), (C) and (D) at $P < 0.005$, but not significant in (E) at $P > 0.1$.

§ See Table 1, footnote ‡.

trypsin.

For separation of the component inducing the polyclonal B-cell activation, living parasites were incubated with trypsin in PBS at room temperature for 10 min. Under light microscope, it was shown that even after tryptic digestion the parasites were not ruptured and their motility was as active as untreated parasites. Electron micrographs of trypsin-treated trypanosomes showed that most of the surface glycoproteins which form a closely packed layer on the plasma membrane were removed without causing any other gross changes in morphology (Fig. 1).

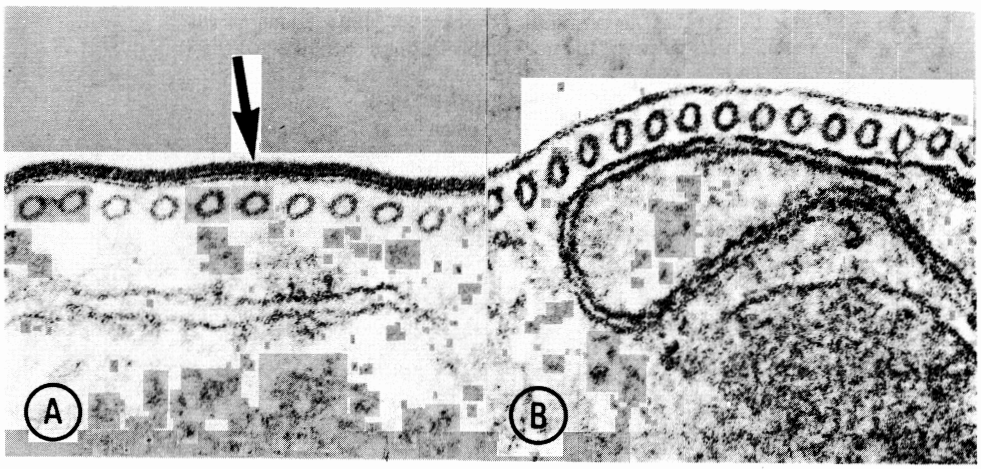

Fig. 1 Electron micrographs of the transverse section of *Trypanosoma gambiense*. (A) control; surface coat (arrow) overlines the plasma membrane of the cell. x 35,000. (B) trypsin-treated; surface coat is uniformly removed from the plasma membrane. x 30,000.

The polyclonal B-cell activation was induced by the 1,000 x *g*-supernatant prepared from trypsin-treated parasites as well as by the cell homogenate of untreated and trypsin-treated parasites (Table 3).

Partial properties of the component responsible for the polyclonal B-cell activation was shown in Table 4. The mitogenic activity of the 1,000 x *g*-supernatant from trypsin-treated parasites was abolished by incubation with pronase or periodate (Table 4, Experiments 1 and 2) and by heating for 10 min at 60°C (Table 4, Experiment 3).

To examine whether an immunosuppressive factor exists in the 1,000 x *g*-supernatant derived from trypsin-treated parasites, the

Table 3 Effect of 1,000 x *g*-supernatant from trypsin-treated *Trypanosoma gambiense* on background anti-SRBC PFC

Fraction*	Anti-SRBC PFC per spleen† ± SE	Stimulation index‡
PBS	160 ± 66 (A)	–
Trypsin	200 ± 58 (B)	1.2
Cell homogenate of untreated parasite	4,340 ± 574 (C)	27.1
Cell homogenate of trypsin-treated parasite	12,410 ± 4,600 (D)	77.6
1,000 x *g*-supernatant from trypsin-treated parasite	4,450 ± 786 (E)	27.8

* Phosphate-buffered saline (PBS), trypsin (50 μg) or trypanosome fractions (50 μg) were injected intraperitoneally.

† Four days after administration of test fractions, background IgM plaque-forming cells (PFC) to sheep red blood cells (SRBC) was measured. Values are arithmetic means, 3–4 mice per group. Difference from (A) was significant in (C), (D) and (E) at $P < 0.005$, $P < 0.05$ and $P < 0.005$ respectively, but not significant in (B) at $P > 0.1$.

‡ See Table 1, footnote ‡.

Table 4 Effect of 1,000 x *g*-supernatant from trypsin-treated *Trypanosoma gambiense* after treating with pronase or periodate and heating on background anti-SRBC PFC

Fraction*	Treatment†	Anti-SRBC PFC per spleen‡ ± SE	Stimulation index§
Experiment 1			
PBS	–	255 ± 87	–
Pronase (20 μg)	–	256 ± 62	1.0
1,000 x *g*-supernatant	PBS	2,924 ± 381 (A)	11.5
1,000 x *g*-supernatant	Pronase (20 μg)	288 ± 50 (B)	1.1
Experiment 2			
PBS	–	80 ± 11	–
Periodate (5 μmoles)	–	204 ± 52	2.6
1,000 x *g*-supernatant	PBS	2,816 ± 528 (C)	35.2
1,000 x *g*-supernatant	Periodate (5 μmoles)	215 ± 102 (D)	2.7
Experiment 3			
PBS	–	72 ± 34	–
1,000 x *g*-supernatant	–	2,636 ± 631	36.6
1,000 x *g*-supernatant	60°C for 10 min	540 ± 258	7.5

* After treatment, phosphate-buffered saline (PBS), pronase (20 μg), periodate (5 μmoles) and 1,000 x *g*-supernatant (50 μg) from trypsin-treated parasites were injected intraperitoneally.

† See materials and methods.

‡ Four days after administration of test fractions, background IgM plaque-forming cells (PFC) to sheep red blood cells (SRBC) was measured. Values are arithmetic means, five mice per group. Difference from (A) was significant in (B) at $P < 0.005$. Difference from (C) was significant in (D) at $P < 0.005$.

§ See Table 1, footnote ‡.

Table 5 Suppression of primary antibody response to SRBC by cell homogenate or 1,000 x *g*-supernatant from trypsin-treated *Trypanosoma gambiense*

Fraction*	Anti-SRBC PFC per spleen† ± SE	Percentage response
PBS	65,600 ± 10,418 (A)	100
Trypsin	47,233 ± 7,563 (B)	70
Cell homogenate of untreated parasite	4,320 ± 1,054 (C)	7
Cell homogenate of trypsin-treated parasite	9,636 ± 3,604 (D)	15
1,000 x *g*-supernatant from trypsin-treated parasite	4,880 ± 1,023 (E)	7

* Phosphate-buffered saline (PBS), trypsin (50 μg) or trypanosome fractions (50 μg) were injected intraperitoneally 4 days before priming with 3×10^7 sheep red blood cells (SRBC).

† Anti-SRBC plaque-forming cells (PFC) was measured 5 days after priming with SRBC. Values are arithmetic means, five mice per group. Difference from (A) was significant in (C), (D) and (E) at $P < 0.005$, but not significant in (B) at $P > 0.1$.

supernatant was injected into mice 4 days before immunization with SRBC. The primary PFC response to SRBC was markedly reduced by this treatment as well as by the injection of the cell homogenate of untreated parasites (Table 5). This immunosuppressive activity of the 1,000 x *g*-supernatant was resistant to pronase and sensitive to periodate (Table 6).

As shown in Table 7, dialysis of the 1,000 x *g*-supernatant from trypsin-treated parasites abolished the enhancement of background anti-SRBC PFC response. However, the suppression of primary antibody response to SRBC was not affected by dialysis, There was not statistical significance between undialyzed and dialyzed 1,000 x *g*-supernatant ($P > 0.1$).

Table 6 Effect of 1,000 x *g*-supernatant from trypsin-treated *Trypanosoma gamiense* after treating with pronase or periodate on suppression of primary antibody response to SRBC

Fraction*	Treatment†	Anti-SRBC PFC per spleen‡ ± SE	Percentage response
PBS	–	106,700 ± 14,633 (A)	100
1,000 x *g*-supernatant	–	4,500 ± 719 (B)	4
1,000 x *g*-supernatant	Pronase	14,400 ± 3,742 (C)	13
1,000 x *g*-supernatant	Periodate	109,400 ± 9,336 (D)	103

* After treatment, phosphate-buffered saline (PBS) or 1,000 x *g*-supernatant (50 μg) from trypsin-treated parasites were injected intraperitoneally 4 days before priming with 3×10^7 sheep red blood cells (SRBC).

† See materials and methods.

‡ Anti-SRBC plaque-forming cells (PFC) was measured 5 days after priming with SRBC. Values are arithmetic means, four mice per group. Difference from (A) was significant in (B) and (C) at $P < 0.005$, but not significant in (D) at $P > 0.1$. Difference from (B) was not significant in (C) at $P > 0.05$.

Table 7 Effect of 1,000 x *g*-supernatant from trypsin-treated *Trypanosoma gambiense* after dialysis on background anti-SRBC PFC and primary anti-SRBC PFC response

Fraction*	Background anti-SRBC PFC per spleen† ± SE	Primary anti-SRBC PFC response per spleen‡ ± SE
PBS	127 ± 30	112,489 ± 16,491
1,000 x *g*-supernatant (undialyzed)	3,451 ± 932 (A)	19,600 ± 7,067 (A')
1,000 x *g*-supernatant (dialyzed)	249 ± 69 (B)	32,950 ± 6,551 (B')

* Phosphate-buffered saline (PBS) or 1,000 x *g*-supernatant (50 μg) from trypsin-treated parasites were injected intraperitoneally.

† Four days after administration of test fractions, background IgM plaque-forming cells (PFC) to sheep red blood cells (SRBC) was measured. Values are arithmetic means, 5–8 mice per group. Difference from (A) was significant in (B) at $P < 0.005$.

‡ Four days after administration of test fractions, 3×10^7 SRBC were injected intraperitoneally. Anti-SRBC PFC response was measured 5 days later. Values are arithmetic means, five mice per group. Difference from (A') was not significant in (B') at $P > 0.1$.

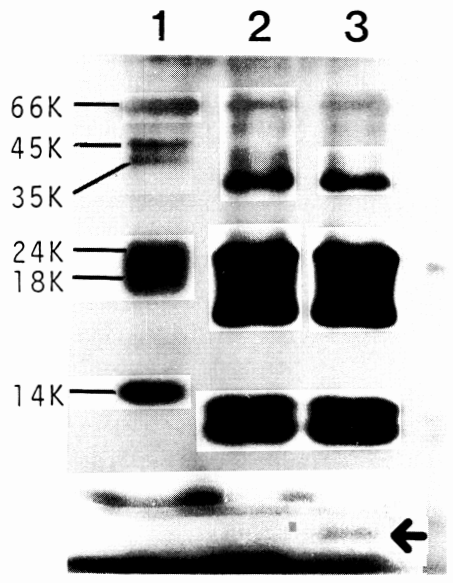

Fig. 2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis of 1,000 × *g*-supernatant from trypsin-treated *Trypanosoma gambiense* and its dialyzed 1,000 × *g*-supernatant. (1) molecular weight markers. (2) dialyzed 1,000 × *g*-supernatant. (3) undialyzed 1,000 × *g*-supernatant, molecular weight of the band (arrow) is approximately 9,000.

The 1,000 × *g*-supernatant and its dialyzed fraction were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to determine the protein composition. The results showed that both undialyzed and dialyzed fractions gave a large number of resolved bands, with an approximate molecular weight of 66,000 to 9,000. Only the 9,000 molecular band was absent after dialysis. All other bands appeared to be unaffected by dialysis (Fig. 2).

Discussion

Both polyclonal B-cell activation and the immunosuppression of primary antibody response to SRBC were successfully induced by injection with a 1,000 × *g*-supernatant derived from trypsin-treated *T. gambiense*. Clayton *et al.* (1979) demonstrated that a crude membrane fraction of trypanosomes induced the polyclonal B-cell activation as well as immunosuppression. Recently, Sacks *et al.* (1982) reported that the partially purified soluble trypanosome fraction was able to induce these immunological modifications only after it was incubated with peritoneal macrophages before transfer into syngenic recipient mice. Because the polyclonal B-cell stimulating activity was periodate sensitive and pronase resistant, and because the lipid extracts from the soluble fraction produced a slight immunosuppressive effect on the antibody response, it was proposed that glycolipid(s) included in a membrane fraction possessed potent mitogenic and immunosuppressive activities. In the present experiment, the polyclonal B-cell stimulating activity was degraded by pronase and periodate treatment. It therefore seems that the carbohydrate molecule(s) is one of the important factors for the elicitation of the polyclonal B-cell activation. It is not clear yet whether the protein molecule(s) is an essential factor itself or a supplemental factor for the initiation of the polyclonal B-cell activation. Alternatively, there is the possiblity that another factor(s) which is required for the B-cell activator is lost by pronase treatment. It is known that variant specific antigen (VSA) forms a closely packed layer visualized in transmission electron microscopy as a surface coat of a dense covering 12–15 nm thick over the entire surface of trypanosomes (Vickerman, 1969; Cross, 1975). Recent work by Diffley (1983) has shown that intravenous injections of the purified VSA in concentrations encountered by the mice infected with trypanosomes elicit the polyclonal B-cell response. In the present study, trypsin treatment of living parasites removed the surface coat without causing any other gross changes in morphology as evidenced by electron microscopic observation. Tryptic cleavage products from VSA are likely to be an important factor for initiation of the polyclonal B-cell activation, although several investigators have reported that the conventionally purified VSA is consistently unable to induce either polyclonal B-cell activation or immunosuppression (Clayton *et al.*, 1979; Sacks *et al.*, 1982).

Reinwald *et al.* (1979) reported the rapid proteolytic degradation of VSA during the isolation process. It would appear that proteolytic enzymes in the cell lysate of parasites may abrogate/reduce the polyclonal stimulating activity. However, several components are present on the surface of trypanosomes other than VSA (Cross, 1984; Beat *et al.*, 1984). It should also be considered whether these surface components are closely associated with the polyclonal B-cell activation.

Of interest in the present study were other findings. The immunosuppressive activity is pronase resistant and periodate sensitive, and remains in the dialyzed solution of the 1,000 × *g*-supernatant from trypsin-treated parasites in spite of the loss of polyclonal B-cell stimulating activity after dialysis. These results seem to indicate that carbohydrate molecule(s) positively relates to the exhibition of the immuno suppressive activity, but that a protein molecule(s) is less important for manifesting the immunosuppressive activity. In addition, the polyclonal B-cell activation and immunosuppression appears to be involved with different materials on the surface of trypanosomes. Support for this explanation is derived from the recent observations that the polyclonal B-cell activation and immunosuppression are uncoupled events in mice subchronically infected with trypanosomes (Baltz *et al.*, 1981), and in mice injected with VSA (Diffley, 1983) or cell homogenate of trypanosomes (Oka *et al.*, 1984).

Injection of the dialysate of 1,000 × *g*-supernatant from trypsin-treated parasites has not yet induced the definite polyclonal B-cell activation. As has been suggested by Sacks *et al.* (1982), the potential for the active component seems to be easily affected and reduced during fractionation process. Further fractionation of the polyclonal B-cell activator and identification of the biologically active fraction to the 9,000 molecular weight proteolytic fragment, which was detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, are currently being attempted.

Summary

A fraction, which was released from the surface of living *T. gambiense* by treating with trypsin and centrifugation, caused the polyclonal B-cell activation and nonspecific immunosuppression of antibody production in mice. When the fraction was treated with pronase or periodate, the polyclonal B-cell activation was abolished. In contrast, the immunosuppressive activity was pronase resistant and periodate sensitive. Although the immunosuppressive activity remained in the dialyzed solution, the polyclonal B-cell activation was abolished by dialysis. Therefore, the polyclonal B-cell activation and the nonspecific immunosuppression were seemingly induced by different components from the surface of trypanosomes.

Acknowledgments

The author thanks Dr. Yoshihiro Ito for helpful suggestions during the course of this study and acknowledges the technical assistance of Miss M. Kuwauchi and Mrs. K. Ohgame.

References

1) Ackerman, B. B. and Seed, J. R. (1976): Immunosuppression during *Trypanosoma brucei gambiense* infections in the field vole, *Microtus montanus*. *Clin. Exp. Immunol.*, 25, 152–158.
2) Assoku, R. K. G., Hazlett, C. A. and Tizard, I. (1979): Immunosuppression in experimental African trypanosomiasis, polyclonal B-cell activation and mitogenicity of trypanosome-derived saturated fatty acids. *Int. Arch. Allergy Appl. Immunol.*, 59, 298–307.
3) Baltz, T., Baltz, D., Giroud, C. and Pautrizel, R. (1981): Immune depression and macroglobulinemia in experimental subchronic trypanosomiasis. *Infect. Immun.*, 32, 979–984.
4) Beat, D. A., Stanley, H. A., Choromanski, L., MacDonald, A. B. and Honigberg, B. M. (1984): Nonvariant antigens limited to bloodstream form of *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense*. *J. Protozool.*, 31, 541–548.
5) Clayton, C. E., Sacks, D. L., Ogilvie, B. M. and Askonas, B. A. (1979): Membrane fractions of trypanosomes mimic the immunosuppressive and mitogenic effects of living parasites on the host. *Parasite Immunol.*, 1, 241–249.

6) Cross, G. A. M. (1975): Identification, purification and properties of clone-specific glycoprotein antigens constituting the surface coat of *Trypanosoma brucei*. *Parasitology*, 71, 393–417.
7) Cross, G. A. M. (1984): Release and purification of *Trypanosoma brucei* variant surface glycoprotein. *J. Cell Biochem.*, 24, 79–90.
8) Diffley, P. (1983): Trypanosomal surface coat variant antigen causes polyclonal lymphocyte activation. *J. Immunol.*, 131, 1983–1986.
9) Goodwin, L. G., Green, D. G., Guy, M. W. and Voller, A. (1972): Immunosuppression during trypanosomiasis. *Brit. J. Exp. Parasitol.*, 53, 40–43.
10) Greenwood, B. M. (1974): Immunosuppression in malaria and trypanosomiasis. In Parasite in the immunized host: Mechanisms of survival, North-Holland, Amsterdam, 137–159.
11) Hudson, K. M., Byner, C., Freeman, J. and Terry, R. J. (1976): Immunosuppression, high IgM levels and evasion of the immune response in murine trypanosomiasis. *Nature (London)*, 246, 256–258.
12) Jerne, N. K. and Nordin, A. A. (1963): Plaque forming in agar by using single antibody-producing cells. *Science (Washington, D.C.)*, 140, 405–406.
13) Lanham, S. M. and Godfrey, D. G. (1970): Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Exp. Parasitol.*, 28, 521–534.
14) Murray, P. K., Jennings, F. W., Murray, M. and Urquhart, G. M. (1974): The nature of immunosuppression in *Trypanosoma brucei* infections in mice. *Immunology*, 27, 815–840.
15) Oakley, B. R., Kirsch, D. R. and Morris, N. R. (1980): A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Anal. Biochem.*, 105, 361–363.
16) Oka, M., Ito, Y., Furuya, M. and Osaki, H. (1984): *Trypanosoma gambiense*: Immunosuppression and polyclonal B-cell activation in mice. *Exp. Parasitol.*, 58, 209–214.
17) Reinwald, E., Rautenberg, P. and Risse, H. J. (1979): *Trypanosoma congolense*: Mechanical removal of the surface coat *in vitro*. *Exp. Parasitol.*, 48, 384–397.
18) Sacks, D. L., Bancroft, G., Evans, W. H. and Askonas, B. A. (1982): Incubation of trypanosome-derived mitogenic and immunosuppressive products with peritoneal macrophages allows recovery of biological activities from soluble parasite fractions. *Infect. Immun.*, 36, 160–168.
19) Vickerman, K. (1969): On the surface coat and flagellar adhesion in trypanosomes. *J. Cell Sci.*, 5, 163–193.

Trypanosoma gambiense 表層由来の画分で誘導されるB細胞のポリクロナール活性化と免疫抑制

岡　三希生
(徳島大学医学部寄生虫学教室)

Trypanosoma gambiense 生虫体をトリプシンで処理し，その遠心上清から得た虫体表層成分に，B細胞のポリクロナール活性化と非特異的抗体産生抑制の作用を認めた．この画分をプロナーゼもしくは過沃素酸で処理すると，B細胞のポリクロナール活性化は消失した．しかし本画分の非特異的免疫抑制の効果は，プロナーゼ処理では影響を受けなかったが，過沃素酸処理はその効果を消失させた．また，本画分を透析するとB細胞の活性化因子は内液から消失し，非特異的免疫抑制の活性のみが認められた．これらのことから，*T. gambiense* の虫体表層に由来する，B細胞のポリクロナール活性化因子と非特異的免疫抑制因子はそれぞれ異なる成分であると考えられた．