# Protective Role of Immune Lymphoid Cells and Phagocytes in Experimental Trichomoniasis in Mice

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In our serial study in experimental trichomoniasis in mice, attempts have been made to elucidate the nature of protective antigen which is thought to be directly related to the induction of specific resistance to protozoan infections.

Mice infected with smaller number of *Trichomonas foetus* than lethal dose were able to survive fatal reinfections (Oka and Osaki, 1963) and an analogous phenomenon was observed in immunizations with microsomes obtained from *T. foetus* cell homogenate (Oka *et al.*, 1967a). Further study (Oka *et al.*, 1967b) suggested that the antigenic informations were localized in ribosomes isolated from microsomes.

From these and another study on serum antibody (Furuya *et al.*, 1972), the protective responses exhibited by the hosts are seemingly of cellular immunity in nature.

This present report is on microscopical observations of immunological protective role of lymphoid cells and phagocytes.

## Materials and Methods

Protozoa: *T. foetus* (Inui strain) were kindly offered by the Department of Protozoology, Research Institute for Microbial Diseases, Osaka University and were made to proliferation in F-bouillon with 10% heat-inactivated bovine serum for 35～40 hours (Oka and Osaki, 1963). Mice inoculated with $3\times10^7$ organisms intraperitoneally were completely fatal. In *in vitro* experiments, the collected parasites were first washed once with Earle's solution containing 0.1% yeast extract and 0.5% lactalbumin hydrolysate and were suspended in the same solution with 20% heat-inactivated calf serum (YLE-C) at the ratio of $10^5$ organisms per ml.

Immunization: Male CF#1 mice of 20～25 g of body weight were immunized with $10^5$ living parasites intraperitoneally and lymphoid cells were derived from axillary, submaxillary and inguinal lymph nodes of the animals 21 days after immunization. After washing with YLE-C solution, the cells were suspended in the same medium being adjusted to $5\times10^5$ cells per ml and the suspension was incubated at 37°C for one hour with an equal quantity of the suspension containing $10^5$ living parasites. Both lymphoid cells and parasites were then collected by centrifugation for light and electron microscopic observations. At the same time, the ascites of immunized mice was withdrawn three hours after intraperitoneal challenges with $3\times10^7$ parasites.

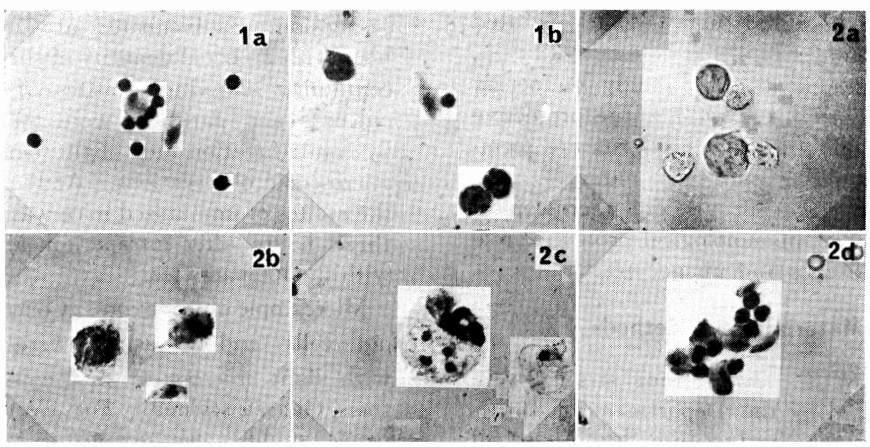
Microscopic observation: Collected lymphoid cells and parasites were examined with light microscope first unstained and then Giemsa stained. For electron microscopic studies, specimens were fixed in 0.05 *M* phosphate buffer, pH 7.2 containing 0.5% glutaraldehyde and after refixed with 2% osmium tetroxide, they were dehydrated in a graded series of aceton prior to the embedding in Epon. Sections were cut on a Porter-Blum microtome and stained with uranyl acetate and lead citrate. Observations were performed at magnifications of 5,000～20,000

by Hitachi HU-11S electron microscope (Hitachi, Ltd.).

## Results

*In vitro*: Lymphoid cells and the parasites which were collected right after the incubation term were examined with light microscope. Up to 50% of the parasites were seen captured by immune lymphocytes swelling and becoming immobile gradually, eventually being led to death on cessation of the protoplasmic movements. This adhesive phenomenon was also observed in approximately 30% of the parasites by contact with nonimmunized lymphocytes but the interaction was only transient and the parasites were freed from the lymphocytes in the meantime. As shown in Figure la, a single immune lymphocyte retained contacts with several parasites in general, whereas being almost one lymphocyte to one parasite in controls (Fig. 1b). Electron micrographs of the adhesion revealed punch-hole destructions of 0.05~0.1 $\mu$ in diameter at the adhesion site of the parasites, and vacuoles were observable in the protoplasm around the destructions (Figs. 3a, 3b and 3d). Furthermore, pleated changes were noticed in the cell membrane of the parasites contacted with immune lymphocytes, but not in controls (Fig. 3c). Larger vacuoles were seen in the parasites in controls and they at large were surrounded by limiting membranes.

*In vivo*: Peritoneal exudates of immune mice challenged with $10^7$ living parasites were examined three hours after challenge with light microscope. The parasites were mostly captured by phagocytes in the exudate, and also lymphocytes, although their number in the exudate was limited, were seen in contact with parasites (Figs. 2a, 2b and 2c). The mode of contact between lymphocytes and parasites was the same as that in *in vitro* experiments (Fig. 2d). Under electron microscope, the parasites were seen surrounded by indented plasma membrane of phagocytes (Fig. 5a), then phagocytized (Fig. 5b), and digested in phagosomes of the cells in the exudate (Figs. 5c, 5d and 5e). In Figure 5a, a single cell envelopes a parasite, while a parasite is surrounded by several cells in Figures 5d




× 1,500

Fig. 1 Oil-immersion photomicrographs showing lymphocytes of an immune mouse in contact with *Trichomonas foetus* (a) and a control (b) being incubated for three hours.

Fig. 2 Oil-immersion photomicrographs of the adhesion between cells in the peritoneal fluid of an immune mouse and *T. foetus* three hours after intraperitoneal inoculation of the parasites into the mouse. Note the macrophages in adhesion (a, unstained; b, Giemsa stained) and in phagocytosis (c, Giemsa stained) and the lymphocytes in contact with the parasites (d, Giemsa stained).

and 5e, and the sweeping manner of mobilized cells in the peritoneal fluid is seemingly rather complicated. The fact that the cell membrane of the parasites in the phagosome of phagocyte vanished suggests that the digestive reaction of phagocytes may initiate at a very early stage of the phagocytotic operation. An immune cell with a large vacuole in the exudate in Figure 5f seems to have completed the digestion. The emergence of eosinophil granules in the vacuole (Fig. 5d) or around the phagocyte (Fig. 5e) suggests that these granules play an eliminating role of foreign substances. Phagocytes in Figures 5c, 5d and 5e are polymorphonuclear neutrophils. The incidence of contacting interactions between lymphocytes and parasites were lower in experiments *in vivo* than *in vitro*, and no abnormalities in the cell membrane of the parasites at their contact sites were detected *in vivo*.

## Discussions

Kelly and Schnitzer (1952) and Schnitzer and Kelly (1953) reported that intramuscular *Trichomonas vaginalis* induced an abscess in mice and that a subsequent inoculation of the parasites at a different region did not cause any abscess and the paraites given disappeared from the site shortly after inoculation. These findings provided an insight into the nature of protective immunity but the fate of the parasites disappeared is remained to be made clear, and features of intramuscular infection are not seemingly satisfactory in ruling out the mode of protection in this sort of survey. In our earlier experiment (Oka and Osaki, 1963), it was confirmed that a large number ($3\times10^7$) of intraperitoneal *T. foetus* resulted in death in mice and that mice given a small number (approximately $10^5$) of parasites acquired a specific resistance and conquered subsequent fatal reinfections. On the contrary, immunization with heat-killed parasites (100°C, 30 minutes) did not induce resistance to fatal infections. A feeling aro se from these findings that the mechanism of protection against protozoan infections was apparently based on cellular immunity, and further studies on experimental trichomoniasis in mice have been conducted from this standpoint of view. Among cell fractions of the parasites, microsomes were found to be the major protective antigen and the most efficient activity was localized in ribosomes especially in ribosomal protein (Oka *et al.*, 1967 a, b; Yamakawa, 1968; Oka *et al.*, 1970). Oka *et al.* (1967 c) reported that intraperitoneally inoculated parasites disappeared from the peritoneal exudate of mice immunized with microsomes or ribosomes of the parasites, and suggested this evidence was to be attributable at least in part to the protective response of the host. From these findings and the fact that sera separated from immunized mice failed to kill the parasites (Furuya *et al.*, 1972), it must be conceded that the protective immune responses in experimental trichomoniasis in mice are of cell-mediated immunity in nature.

This present report is on interactions between the parasites and host cells hoth *in vivo* and *in vitro* in the absence of humoral antibodies using light and electron microscopes.

In *in vitro* experiments, immune cells obtained from the lymph nodes of immunized mice captured and killed the parasites affecting them in a direct way. This direct seizing activity of lymphoid cells in the absence of humoral factors is to be interpreted as protective and cellular. Although destructions of the cell membrane of the parasites observed under electron microscope could be mediated by cell-binding antibody, it was quite clear that the destruction was by no means originated in humoral factors.

The swelling and death of the captured parasites by immune lymphoid cells might be due to the unbalance of osmotic pressure inside the cytoplasm of the parasites caused by punch-hole openings on the membrane.

The primary purpose of *in vivo* experi-

ments with respect to phagocytes was to know how those phagocytes in the peritoneal fluid could have managed trichomonads which are more than ten times as large as bacteria in size, and the appearance of eosinophil granules in vacuoles of the phagocytes, polymorphonuclear neutrophils, stimulated a new interest suggesting a closely related contribution of the granules to "infection".

The application of Freund's complete adjuvant induced migrations of macrophages into peritoneal fluid in experimental toxoplasmosis in mice and resulted in enancement of specific resistance to reinfection (Oka *et al.*, 1969). Mice intensely immunized with microsomes or ribosomes were able to conquer the challege of fatal infection with the trichomonads and the challenged parasites disappeared from the peritoneal cavity shortly after challenge (Oka *et al.*, 1967 c).

The above-mentioned phenomena were taken place in the peritoneal cavity of mice and, as was shown in *in vitro* study in this report, similar protection activites were to be developed in certain other tissues and organs. Thus, activated lymphocytes and macrophages are to be counted in important members of protection immunity against protozoan infections.

## Summary

Lymph node cells derived from immunized mice with living *Trichomonas foetus* were capable of capturing and killing the parasites in *in vitro* experiments, and destructions were observed in the parasites at the site of contact with the immune lymphocyte under electron microscope. The contact phenomenon was also demonstrable but only transient between lymphocytes derived from nonimmune mice and the parasites, and neither impediment in mobility nor destructions in the contacting region of the parasites were observed.

Three hours after intraperitoneal inoculation of *T. foetus* into immune mice, the mouse ascites was examined with electron microscope and a number of phagocytes were seen migrating into the peritoneal fluid and then capturing, phagocytizing and digesting the parasites. Eosinophil granules were present in the phagosomes of phagocytes suggesting a possibility of playing a preventing role against invading micropathogens.

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## 実験トリコモナス症における免疫リンパ細胞と食細胞の防御的役割

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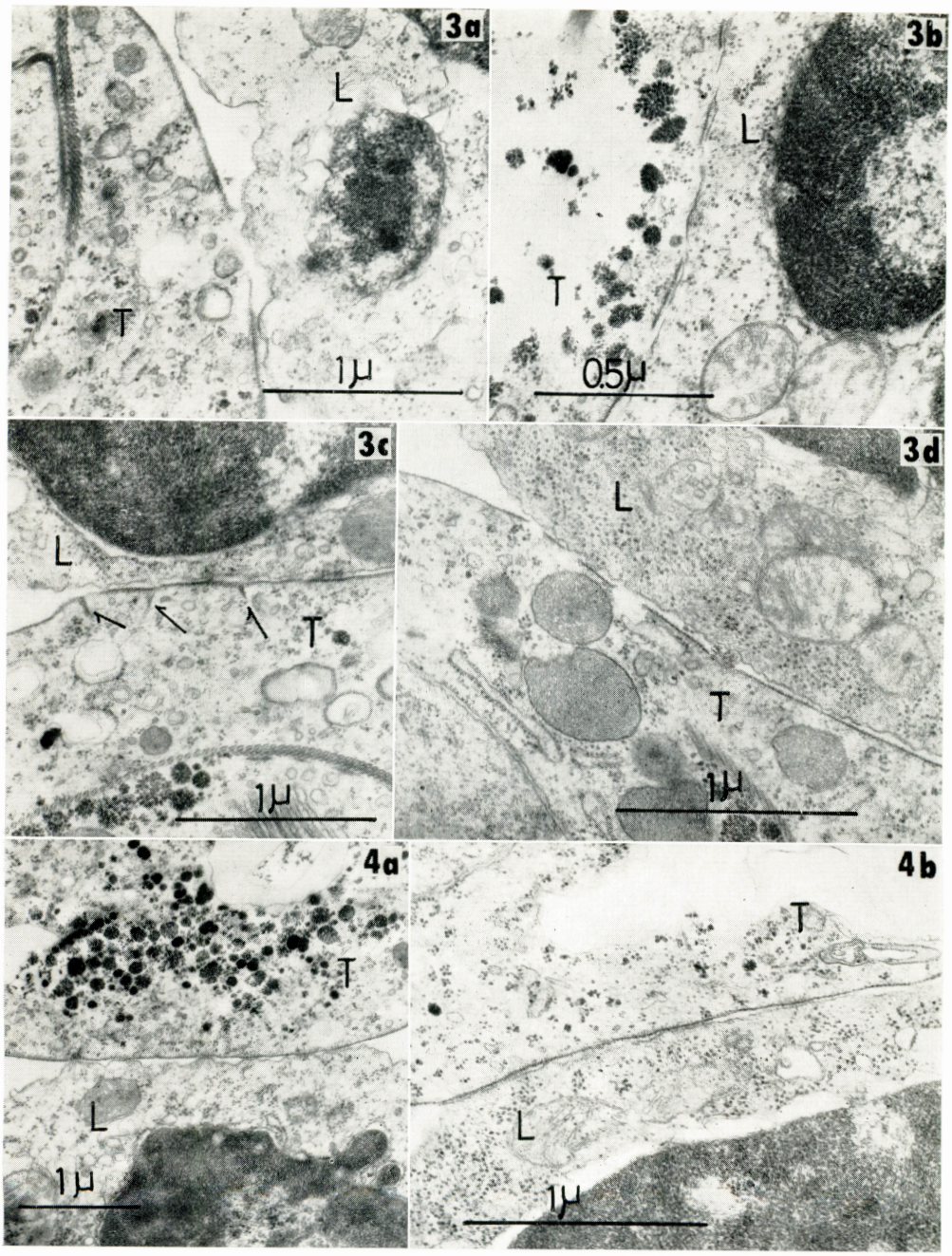
実験トリコモナス症において，生原虫（*Trichomonas foetus*）で免疫したマウスのリンパ節細胞は *in vitro* で原虫を捕そくし死に至らしめた．免疫リンパ細胞と原虫との接着面の電子顕微鏡観察で，原虫細胞膜側のみに数カ所の破損を認めた．無処置リンパ細胞と原虫の場合，接着現象を認めたが接着は一時的で原虫の活動性に影響せず，原虫細胞膜の損傷は見られなかつた．

免疫マウスの腹腔内に *T. foetus* を接種し，3時間後の観察で，多数遊出した食細胞が原虫を捕そくし，続いてどん食消化する過程を電子顕微鏡で認めた．同時に好酸球性か粒が食細胞内に認められ，侵入した病原体に対し好酸球が直接何らかの役割を果たすことが示唆された．




Figs. 3 and 4 Electron photomicrographs showing the adhesion between immune mouse lymphocytes (L) and *T. foetus* (T). A certain number of punch-hole destructions on the limiting membrane of the parasites (3 a, b and d) and pleated changes in the membrane (3 c) are demonstrated except for controls (4 a and b).

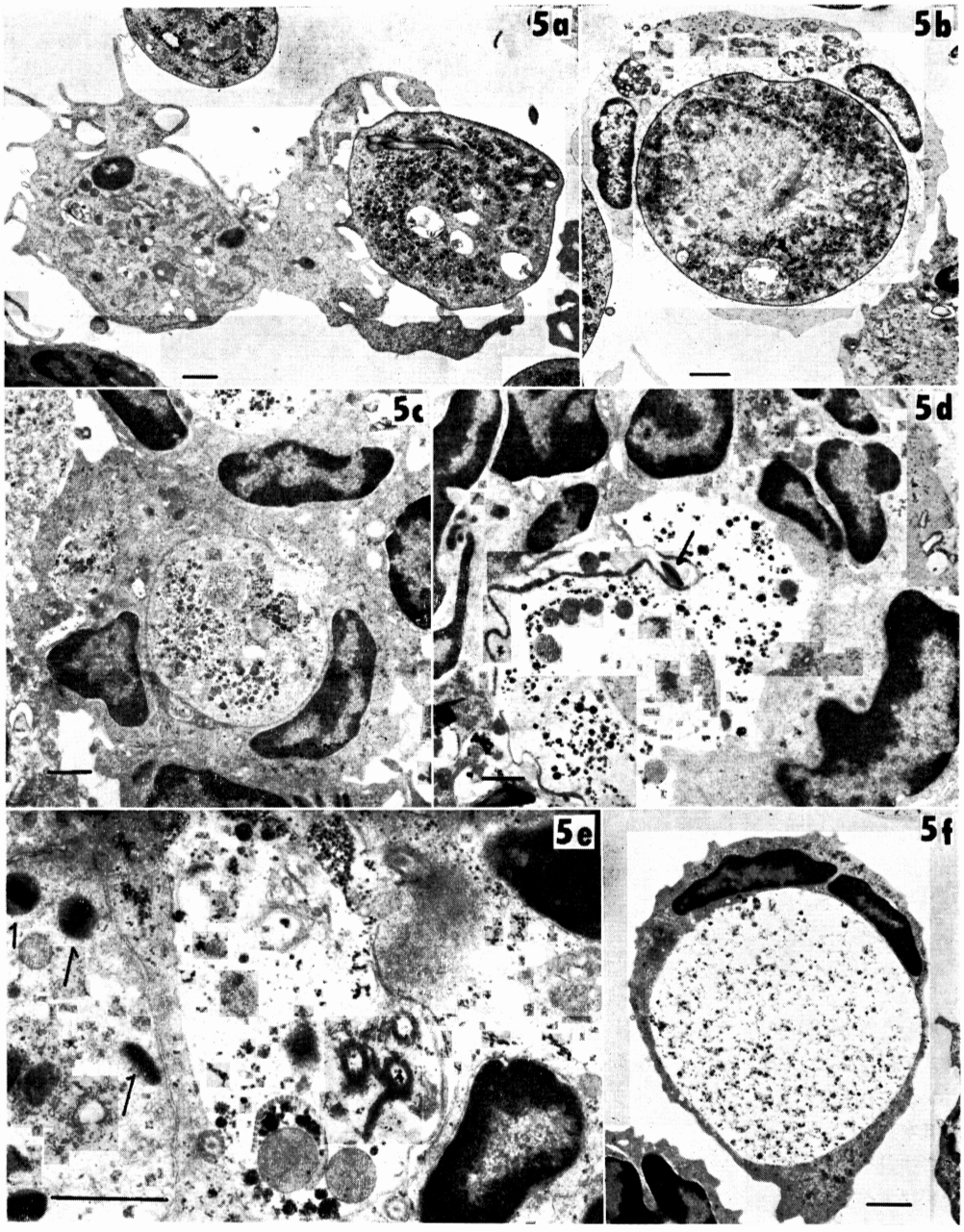



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Fig. 5 Electron photomicrographs of phagocytes in the peritoneal fluid of an immune mouse three hours after inoculation of *T. foetus*. It is to be noted that a parasite is seen first captured by a phagocyte at the site of its indented membrane (a) and then being proceedingly phagocytized (b~e). A large phagosome of the phagocyte is depicted (f) and eosinophil granules are indicated by arrows (d and e).