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# STUDIES ON PHAGOCYTOSIS OF PROTOZOAN *TRICHOMONAS VAGINALIS* BY MURINE MACROPHAGES

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**Shih-Rong Wang and David Chao** (1987) Studies on phagocytosis of protozoan *Trichomonas vaginalis* by murine macrophages. *Bull. Inst. Zool., Academia Sinica* **26**(4): 249-255. This investigation was designed to study the effects of various sources of sera and peritoneal exudate cells on phagocytosis of *Trichomonas vaginalis in vitro*. Peritoneal macrophages from immunized or normal mice were employed in this study. *T. vaginalis* was first incubated in 10% FCS-RPMI1640 culture medium containing different dilutions of mouse antiserum or normal serum and then added to the macrophages cultures. The *in vitro* phagocytic activity was measured at 2, 4, 8 and 12 hours along the macrophage-trichomonad interaction. Our data showed that the phagocytic capability of sensitized macrophages was higher than that of normal mouse macrophages. The normal serum from male mice enhanced the phagocytic activity of sensitized and normal macrophages in the early stage of cultivation, while the immune serum enhanced in the late stage of cultivation. The normal serum from female mice showed little opsonic activity in this study.

The protozoan *Trichomonas vaginalis* is a common cause of vaginitis (Honigberg, 1978). It has been reported that cell-mediated immunity (CMI) plays a more important role in immunity to *T. vaginalis* infection (Michaels and Rogers, 1971), although the trichomonacidal effects by CMI are not clearly understood. It has been reported that some factors of *T. vaginalis* were chemotactic for polymorphonuclear leucocytes (PMNs) (Mason and Forman, 1980; Rein *et al.*, 1980; Mason and Forman, 1982). Phagocytosis of *T. vaginalis* by host cells could be demonstrated as the following events. Landolfo *et al.* (1980) and Mantovani *et al.* (1981) demonstrated that *in vitro* cell-mediated cytotoxicity (CMC) against the extracellular protozoan *T. vaginalis* exist in both murine and human system. The effector cells in these systems belong to monocyte-macrophage series. It has also been known that in either human or murine case, significant differences in infection rate and symptoms exist between the male and the female (Landolfo *et al.*, 1981). The reasons are still not clear. In this investigation, the relationship between the sera and peritoneal macrophages from various sources on the *in vitro* phagocytosis of *T. vaginalis* were further evaluated.

## MATERIALS AND METHODS

### Parasites

The strain of *Trichomonas vaginalis* was originally isolated from the genital tract of a patient attending the Gynecologic and Obstetric Clinics of Veterans General Hospital, Taipei in 1980. It was cultured in Locke's medium containing 5% horse serum in screw-capped culture tubes at 37°C and maintained

by subinoculation into fresh medium at 3-day intervals. Cloning of parasites by limiting dilution method was performed in 96-well microplates. Wells containing only one trophozoite were labled for further passages in fresh Locke's medium, the pure cultured organisms were enriched. They were then transferred to 10 ml culture medium for multiplication. After 72 hours cultivation, they were harvested and washed with 25 mM phosphate-buffered saline, pH 7.2 (PBS) by centrifugation at 300×g for 10 min and then resuspended in PBS. Hemacytometer counts were made of the parasites after they were washed three times in PBS.

**Animals**

BALB/c mice employed in this study were obtained from animal breeding facility, National Yang-Ming Medical College. They were housed in plastic cages and given food and water *ad libitum*. Mice used in the experiments were two to three months old and weighed approximately 20 gm.

**Sera**

Female mice were immunized intraperitoneally with 0.2 ml parasite suspension containing $2\times10^6$ trichomonads. Challenge inoculation was carried out two weeks later with the same inoculum. Blood were collected aseptically by cardiac puncture four days after the challenge infection. Normal sera were obtained from male and female mice. All the serum samples were heat-inactivated at 56°C for 30 min to abolish complement activity and stored at −20°C until being used.

**Macrophages**

Peritoneal exudate cells were collected from normal and immunized mice by the following procedures. Mice were killed by ether anesthesia, then injected intraperitoneally with 5 ml of RPMI 1640 medium. The peritoneal fluids were collected after gentle abdominal massage. The macrophage-rich suspension was obtained and stored on ice bath. Approximately $2\times10^5$ cells in 1 ml RPMI 1640 medium containing 10% fetal calf serum (FCS) were seeded onto a 13 mm diameter cover slip in each well of 24-well tissue culture plate. The plate was incubated at 37°C for 24 hours in a 5% $CO_2$ humidified atmosphere to allow the cells to settle and adhere to the cover slips. Non-adherent cells were removed by dipping the cover slips into RPMI 1640 medium three times. The cover slips were transferred into a new plate with fresh culture medium.

**Phagocytosis test**

Motile trichomonads were collected and resuspended in RPMI 1640 medium containing 10% FCS. Two-fold serial dilutions of sera were performed in the culture medium from 1:10 to 1:80. Macrophages in each well were incubated with $1\times10^5$ motile trichomonads. After 2, 4, 8 and 12 hours incubation. Cover slips were removed from the the wells, washed three times, and stained with Giemsa solution. Then count the percentage of phagocytosis in *T. vaginalis*-macrophage cocultivation.

## RESULTS

*Trichomonas vaginalis* was phagocytized by both sensitized and normal macrophages (Fig. 1). However, phagocytic activity of sensitized macrophages was always higher than the normal macrophages, irrespective of the source or dilution of the serum and the incubation time (Fig. 2-4).

Results of sensitized macrophages incubated with trichomonads in 1:10 diluted immune serum, normal serum from male mice and female mice were illustrated in Fig. 5. The phagocytic activity of sensitized macrophages was significantly higher when normal serum from male mice was present during the first 4 hr of incubation. At the 8th hr of incubation, the enhancement ability of immune sera was the highest. There was no significant difference among the addition

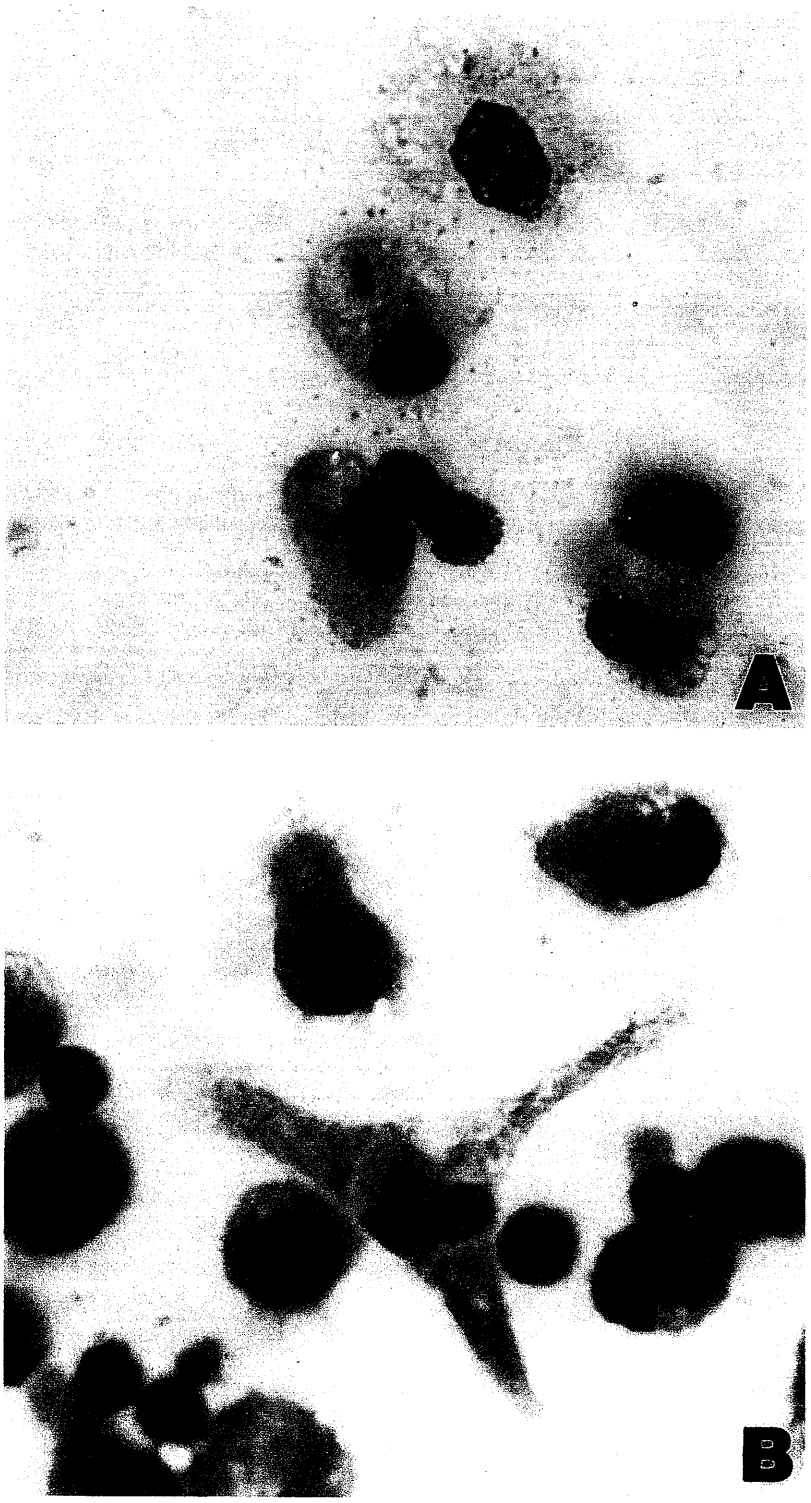


Fig. 1. Phagocytosis of *T. vaginalis* by normal macrophage (A) and immune macrophage (B)

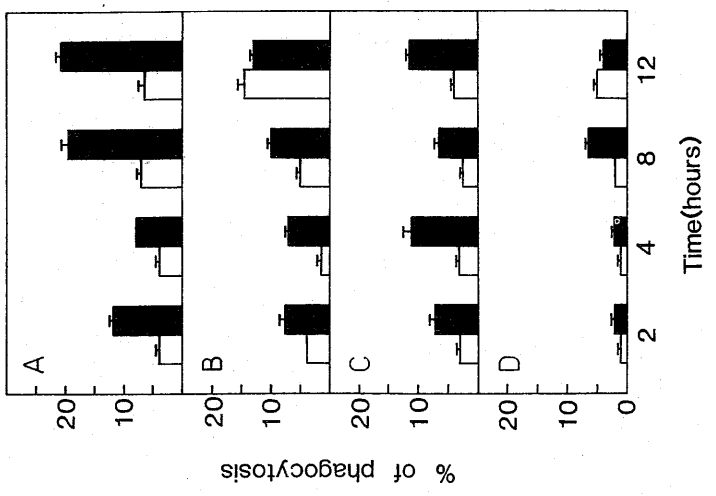


Fig. 2. Phagocytosis of *T. vaginalis* by normal macrophages (□) and immune macrophages (■) in the presence of immune serum.
(A 1: 10, B 1:20, C 1:40, D 1:80)

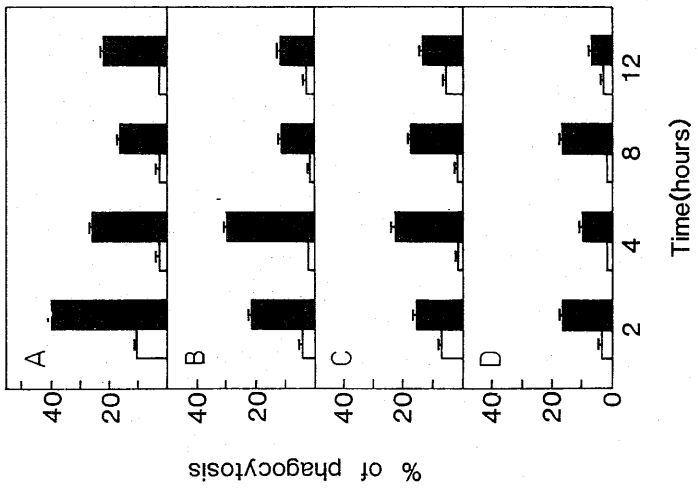


Fig. 3. Phagocytosis of *T. vaginalis* by normal macropahges (□) and immune macrophages (■) in the presence of normal sera from male mice.
(A 1:10, B 1:20, C 1:40, D 1:80)

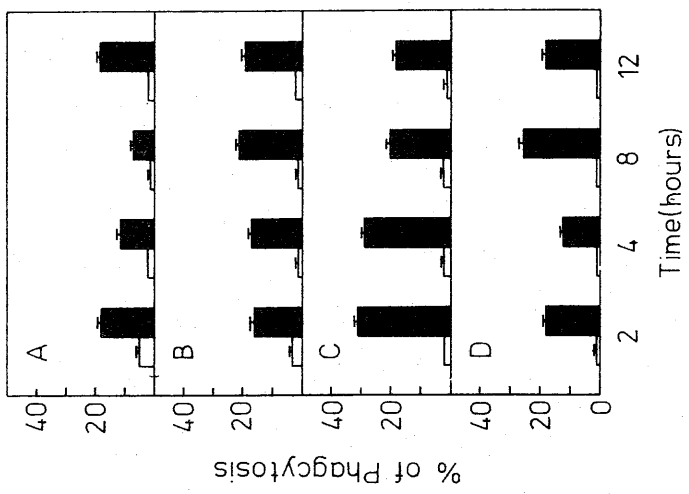


Fig. 4. Phagocytosis of *T. vaginalis* by normal macrophages (□) and immune macrophages (■) in the presence of normal sera from female mice.
(A 1:10, B 1:20, C 1:40, D 1:80)

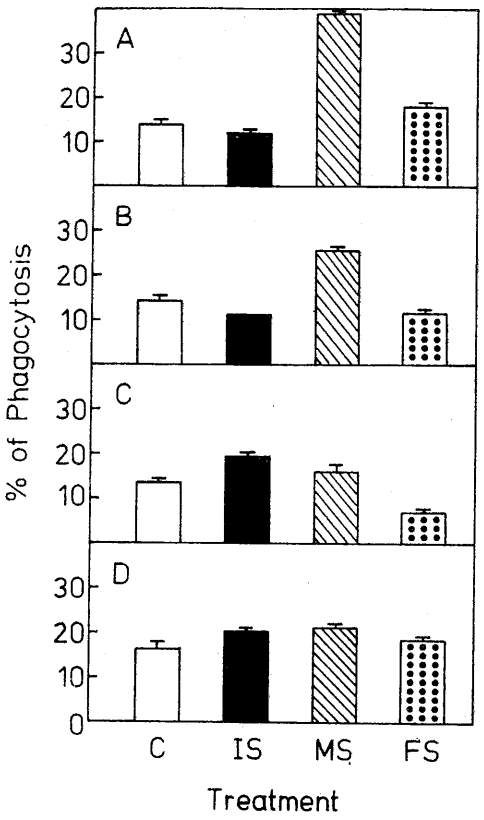


Fig. 5. Comparison of phagocytosis of *T. vaginalis* by immune macrophages in the presence of immune serum (IS), male normal serum (MS), female normal serum (FS) and culture medium control (C) after 2 hr(A), 4 hr (B), 8 hr (C) or 12 hr (D) incubation.

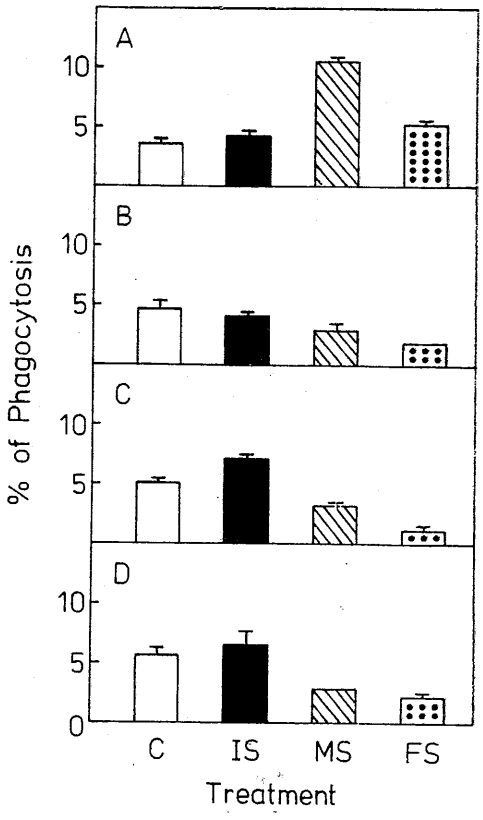


Fig. 6. Comparison of phagocytosis of *T. vaginalis* by normal macropahges in the presence of immune serum (IS), male normal serum (MS), female normal serum (FS) and culture medium control (C) after 2 hr (A), 4 hr (B), 8 hr (C) or 12 hr (D) incubation.

of immune serum, normal serum, and the control group after 12 incubation with normal macrophages were similar to that of sensitized macrophages Fig. 6. The phagocytic activity of normal macrophages was the highest in the addition of normal serum from male mice after 2 hr of incubation. There was no significant difference between the addition of immune serum, normal serum from female mice and the control group. However, the phagocytic activity was lowest in the presence of normal serum from female mice after 4 hr incubation. The immune serum exhibited greater opsonin activity at 8 hr of incubation. These was no significant difference between immune serum treated group and the control group after 12 hr incubation.

## DISCUSSION

*Trichomonas vaginalis* is a common sexually transmitted disease responsible for urogenital symptoms in both man an l woman (Honigberg, 1978). The phagocytosis of this extracellular parasite is not studied as extensively as other invasive or facultative intracellular protozoa. *T. vaginalis* may be killed by cytotoxicity or soluble factors of macrophages (Landolfo *et al.*, 1980; Mantovani *et al.*, 1981; Martinotti *et al.*, 1983). These trichomonacidal effects may be important as a defense mechanism in a host

infected with *T. vaginalis*.

In this study, we have demonstrated that phagocytosis of *T. vaginalis* could be enhanced by the addition of serum. However, the enhancement effect may be achieved through different pathways. The opsonization activity of normal serum from male mice was significant in the early stage of incubation, that of immune serum was in the late stage of incubation, while the normal serum from female mice showed little activity in this study. Our results also showed that phagocytosis by sensitized macrophages was stronger than that by normal macrophages. There is evidence that normal macrophages mediated adherence or phagocytosis by carbohydrate-based opsonic system (Brown and Benacerraf, 1984). Activated macrophages differ from normal mactophages in that they lack receptors for the carbohydrate on opsonins, but mediated largely by specific antibody and Fc receptor. To the normal macrophages, it is possible that there are some carbohydrate coating opsonins in normal serum of male mice such as fibronectin, alpha-2-macroglobulin, etc. (Brown and Kreier, 1986). Whereas these opsonins might be unstable, so the stimulating effect could not persist. Alternately, there are not only carbohydrate-coating opsonins but also high concentration of macrophage activating factor (MAF) in immune serum (Hibbs *et al.*, 1977). Besides, the interaction between macrophages and immune serum was time-dependent, so the phagocytosis was enhanced in the late stage.

In the case of sensitized macrtophages, normal serum may contain nonspecific antibodies and their effect could not persist to late stage of incubation by the same reason described above. Immune serum contains antibodies which could stimulate the phagocytosis specifically. Heat-labile components of immune serum had been inactivated so the opsonin effect was delay to the late stage. These results indicated complements might play an important role in macrophage-trichomonad interaction. It has been confirmed *in vitro* in the *Trypanosoma lewisi* infection (Lange and Lysenko, 1960).

The effect of serum on macrophage-trichomonad interaction was examined for up to 96 hours in an earlier preliminary test. We found that the immune serum always had stronger stimulating effect than normal serum. The effect was significant since the 8th hour of incubation, and was the greatest at the 12th hour. In this study, we demonstrated that phagocytosis dose play an active and essential role in the control of *T. vaginalis* infection and sera from different sources may be effective at different stages of the infection.

When BALB/c mice were infected with *T. vaginalis*, males were more resistant than females. Besides, if there is any symptom in the male, it is always mild. In this study, we found that the stimulating effect of normal serum from male mice was greater than that of female. Landolfo *et al.* (1980) suggested that it might be correlated with the differences of metabolites or hormones present in different sexes. It is possible that female mice had weaker cellular immunity to this parasitic flagellate infection, just like what has been described in the *Leishmania tropica* infection (Giannini, 1986). In addition to hormone factors, infection may also be affected by genetic factors (Loh and Baltimore, 1984; Michael and Rongers, 1971). It would be worthy to search for the mechanism by which different sex exhibit different pathogenecities in *T. vaginalis* infection.

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# 體外培養小白鼠巨噬細胞對陰道滴蟲 (*Trichomonas vaginalis*) 之吞噬作用

王仕蓉　趙大衛

本研究之目的是在探討體外培養中各種不同來源的血清對小白鼠巨噬細胞吞噬陰道滴蟲的影響。取正常及被免疫的 BALB/C 雌鼠之腹腔巨噬細胞與免疫血清、雌雄鼠正常血清、依 1：10、1：20、1：40 及 1：80 的稀釋濃度，與陰道滴蟲在體外共同培養，經 2、4、8 及 12 小時培養後，分別取出並染色觀察。結果顯示，被免疫活化之巨噬細胞對陰道滴蟲的吞噬性較正常巨噬細胞强。雄鼠正常血清及免疫血清對吞噬作用皆具影響力，前者在培養前期有較顯著的促進作用，後者則在培養後期才顯現出增强作用；而雌鼠正常血清則少有促進作用。由此可知各血清影響吞噬作用的效果不同，可能與雌雄性別耐受性不同有關，且也可進一步評估巨噬細胞在陰道滴蟲感染時所扮演的角色和地位。