[Jap. J. Parasit., Vol. 26, No. 3, 113-115, 1977]

Separation of *Toxoplasma* Tachyzoites by Filtrating Peritoneal Exudate of Infected Mice Through Cellulose Powder

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(Received for publication ; March 23, 1977)

Introduction

Purification of *Toxoplasma* tachyzoites without contamination of host cells is a prerequisite procedure for studying metabolism, interaction with host cell and antigen preparation of the parasite. A variety of attempts have been, so far, reported to obtain free parasites by using glass filter (Fulton and Spooner, 1957), gauze filter (Lycke and Lund, 1964) or sonic vibration (Tsunematsu, 1960). But the number of the parasites recovered by those methods were found to be extremely reduced. Although zonal density gradient centrifugation method was reported to give a high recovery of pure parasites (Masihi *et al.*, 1976), it requires complicated several steps of procedure and much expenses. Since it has been known that satisfactory removal of white blood cells can be achieved by passing blood through cellulose powder filter (Homewood and Neame, 1976), we examined if this filter can be used or not as an instrument to separate the free parasites from peritoneal exudate of infected mice.

Materials and Methods

The virulent RH strain of *Toxoplasma gondii* was used. The strain was maintained by serial passage of peritoneal exudate of infected mice into male ddY mice at 3-4 days intervals. Three days after inoculation with the parasites, mice were killed by chloroform and administered intraperitoneally with 4 ml of phosphate-buffered saline (PBS), pH. 7.2, containing 0.02% EDTA. The peritoneal exudate collected by pipette was diluted with 0.02% EDTA-PBS to a suspension containing 2×10^7 tachyzoites per ml, in which about 2×10^6 peritoneal leukocytes were contained.

Adequate volume of cellulose powder (CF-11, Whatman) suspended in 0.02% EDTA-PBS was poured into glass tube (7 mm, diameter), the bottom of which was packed with small quantity of cotton wool. After the suspension medium, EDTA-PBS, was run through off, 2 ml of the exudate sample was then overlaid on top of the prepared column at room temperature. After the sample had passed into the column, 0.02% EDTA-PBS was run through the column. Tachyzoites and peritoneal cells in the eluted suspension were counted in a hemocytometer. Viability and infectivity of the parasite were checked by trypan blue dye exclusion test (Boyse *et al.*, 1964) and mice subinoculation test, respectively.

Results

When 2 ml of the exudate suspension was overlaid onto 1 ml of wet cellulose powder (1 ml-column), about 80% recovery of the parasite was found in 5 ml of the filtrate, which contained less than 3% of peritoneal leukocytes of original overlay (Fig. 1a). Host cell contamination was diminished to 0.2%

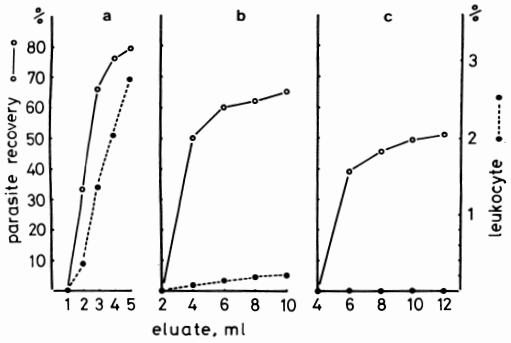

Fig. 1 Separation of *Toxoplasma* tachyzoites by passing peritoneal exudate of infected mice through cellulose powder. 2 ml of the exudate was overlaid on top of the wet powder of volume, a) 1 ml, b) 2 ml, or c) 4 ml. O——O, per cent of the parasite recovery, and ●---●, per cent of leukocyte contamination in the eluate.

in the case of 2 ml-column, in total filtrate 65 % of the parasite being recovered (Fig. 1b). Nearly complete removal of the leukocytes (less than 0.01 %) was accomplished by the usage of 4 ml-column but concomitantly about a half of the total parasites were lost (Fig. 1c). A low recovery of the parasites was also found when the original overlay had contained more than 2×10^6 peritoneal leukocytes per ml of the exudate regardless of the volume of cellulose powder.

The recoved parasites seemed to be intact morphologically. More than 99 % of the parasites were shown to possess the viability by dye exclusion tests both before and after filtration through cellulose powder. As the test for the infectivity of the parasites, 10^5 or 10^3 tachyzoites from both filtrated and untreated exudate were inoculated intraperitoneally into 2 month-old male ddY mice. Difference in mortality of infected mice between two groups was not observed (Table 1).

Table 1 Effect of the filtration of the exudate on the viability of RH *Toxoplasma* tachyzoites as determined by inoculation of mice

Inoculum size	Day after inoculation	No. mice dead/No. inoculated	
		filtered	untreated
1×10^5	6 day	$\frac{3}{5}$	$\frac{2}{5}$
	7 day	$\frac{5}{5}$	$\frac{5}{5}$
1×10^3	8 day	$\frac{2}{5}$	$\frac{2}{5}$
	9 day	$\frac{5}{5}$	$\frac{5}{5}$

Discussion

Bio-medical research of obligatory intracellular parasite, *Toxoplasma gondii*, has been quite limited. The reason seems to be mainly due to a lack of useful tool to separate the parasite from host cells. Recenty Masihi *et al.* (1976) have reported that zonal density gradient centrifugation of the peritoneal exudate of infected mice yielded a high recovery of the parasite accompanying with marked decrease in number of leukocytes, less than 1 % of the initial level. Filtration of the exudate through cellulose powder, in the present study, gave better results than those obtained by the a forementioned investigators. About 80 % of original number of the parasites were recovered by the filtration when the exudate was applied to the cellulose powder at a ratio of 2 volume of the exudate to one volume of cellulose powder and 65 % of them were recovered at a ratio of 1 : 1. and leukocytes contaminations were 3 % and 0.2 % of original oyerlay, respectively. Nearly complete removal of leukocytes could be achieved at a ratio of 1 : 2, though a half of the parasites were lost. Thus, the use of cellulose powder gave good results on the recovery and the purification of parasites. Furthermore, this method has another advantages, *i. e.* simplicity, unexpensiveness and no-requirement of much time. It is thought that the tachyzoites of *Toxoplasma gondii* may be separated very easily from other ingredients in the peritoneal exudate by employing this method, in expectation of the application for immunological or biochemical investigations.

Summary

Separation of *Toxoplasma* tachyzoites from mouse peritoneal exudate was tried by using cellulose powder. Filtration of the exudate through it gave nearly complete removal of host white cells and high recovery of the parasite. Since this method is also simple and unexpensive and does not require much time, it is useful for purification of the parasites.

Acknowledgements

Financial support of this work was given in part by Research Grant from the Ministry of Education, Japan. We wish to thank Mr. S. Waki, Dept of Parasitology, Gunma University, for his discussion.

References

1) Boyse, E. A., Old, L. J. and Chouroulinkov, I. (1964) : Cytotoxic test for demonstration of mouse antibody. Methods in Medical Res., 10, 39-47.
2) Fulton, J. D. and Spooner, D. F. (1957) : Preliminary observation on the metabolism of *Toxoplasma gondii*. Trans. Roy. Soc. Trop. Med. Hyg., 51, 123-124.
3) Homewood, C. A. and Neame, K. D. (1976) : A comparison of methods used for the removal of white cells from malaria-infected blood. Ann. Trop. Med. Parasitol., 70, 249-251.
4) Lycke, E. and Lund, E. (1964) : A tissue culture method for titration of infectivity and determination of growth rate of *Toxoplasma gondii*. I. Acta Path. Microbiol. Scand., 60, 209-220.
5) Masihi, K. N., Mach, O., Valkoun, V. and Jira, J. (1976) : *Toxoplasma gondii* : Large scale purification by zonal density gradient centrifugation. Exp. Parasit., 39, 84-87.
6) Tsunematsu, Y. (1960) : Purification of *Toxoplasma* by means of sonic vibration and tryptic digestion. Am. J. Trop. Med. Hyg., 9, 556-561.

セルロースパウダー濾過によるトキソプラズマ遊離原虫の分離

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トキソプラズマ RH 株感染マウスの腹水より宿主細胞を十分に除去し，遊離原虫を得る方法として，セルロースパウダーの濾過法が適するかどうかを検討した．その結果，濾過される腹水量とセルロースパウダーの量の比率を変えることにより，遊離原虫を効率よく回収（80％）することができ，白血球の混入もほぼ完全（0.01％以下）に防ぐことができた．