[Jpn. J. Parasitol., Vol. 45, No. 6, 552–555, December, 1996]

Research Note

Intracellular Existence: A Prerequisite for Long Term Preservation of *Toxoplasma gondii* Tachyzoite

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(Accepted December 27, 1996)

Abstract

The condition for cryopreservation of *Toxoplasma gondii* was investigated. Experience on long term preservation of malaria parasite led to the examination of the difference between peritoneal exudate from mice infected 35 and 42 hrs previously which included substantial number of infected cells and from that infected 4 days previously in which most of the toxoplasma are free tachyzoites. The former materials were placed in polypropyrene freezing tubes with glycerol and stored in deep freezer (–80°C) for more than a year. Long term preservation using the latter materials failed. Thus, the use of peritoneal exudate at proper time of infection, when many peritoneal cells are infected, seems to be the key factor for cryopreservation purpose.

Key words: *Toxoplasma gondii*; tachyzoite; cryopreservation; intracellular parasite.

Tachyzoites of *Toxoplasma gondii* are very difficult to be cryopreserved under extracellular environment. So far, peritoneal exudate from infected mice has been used for long term storage of this protozoa, but still, intracellular parasites are said to fare very poorly during freezing (David *et al.*, 1994). *Toxoplasma* mainly obtained from mouse peritoneal exudate (David *et al.*, 1994; Mackie, 1972; Samantaray *et al.*, 1980) or from cultured cells (Bollinger *et al.*, 1974; Smith, 1973) has been utilized for cryopreservation purpose. Preservation of this protozoa in mouse peritoneal exudate by deep freezing has also been tried but without success.

In contrast, long term preservation of malaria parasites, *Plasmodium falciparum* and *Plasmodium berghei* has been easy in our laboratory using glycerol as cryoprotectant. Intracellular existence of *Toxoplasma* might be the key factor for long term preservation of this parasite. The successful outcome of this experiment are as follows: Twenty ddY mice were inoculated with *Toxoplasma* RH strain according to conventional peritoneal inoculation. After 35 and 42 hrs of infection, peritoneal cells were collected using RPMI-1640. The cell suspension was centrifuged at 1,500 rpm for 10 min, supernatant was decanted, then 10 ml of RPMI-1640 containing 10% mouse serum as well as same volume of freezing solution (Glycerol 35 g, D-solbitol 3 g, NaCl 0.65 g/distilled water 100 ml) was added and mixed thoroughly. The mixture was dispensed in aliquots of 0.6 ml into polypropyrene freezing tubes (Falcon freestanding cryogenic vial, Becton Dickinson and Company, Franklin Lakes, N. J., U. S. A.). The tubes were kept in a deep freezer (–80°C) until use.

The materials were reconstituted from the frozen state by putting the tubes into a beaker of water at 37°C and shaking. The thawed material was inoculated into mouse peritoneal cavity at 0.3 ml each and then the mouse was examined to confirm infection by detecting parasites in peritoneal exudate and observing the mortality of animals.

Figure 1 shows the survival time of mice inoculated with preserved peritoneal exudate from mice, which were obtained at 35 hrs after infection of *Toxoplasma* RH strain. Just after peritoneal wash-

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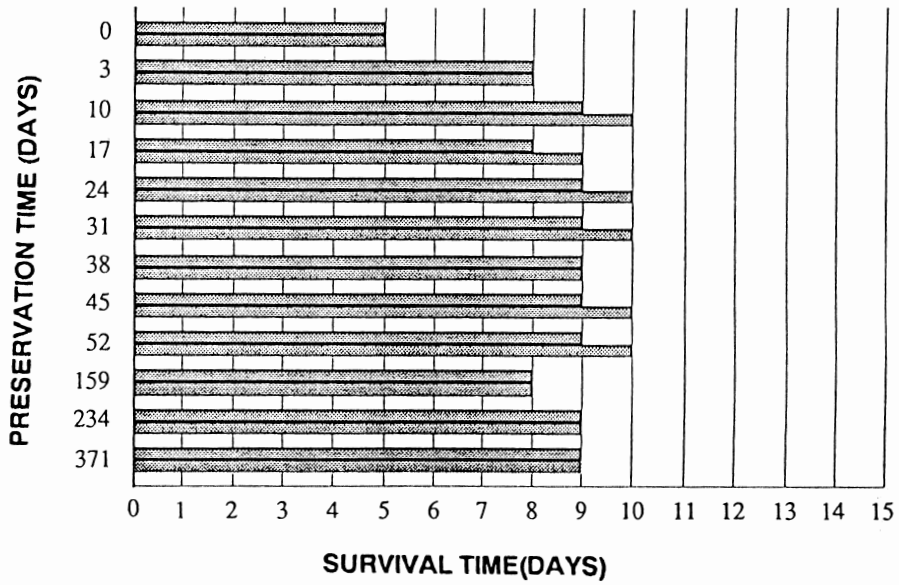

Fig. 1 Survival period of mice inoculated with preserved peritoneal exudate obtained from mice at 35 hr after infection of *Toxoplasma* RH strain.

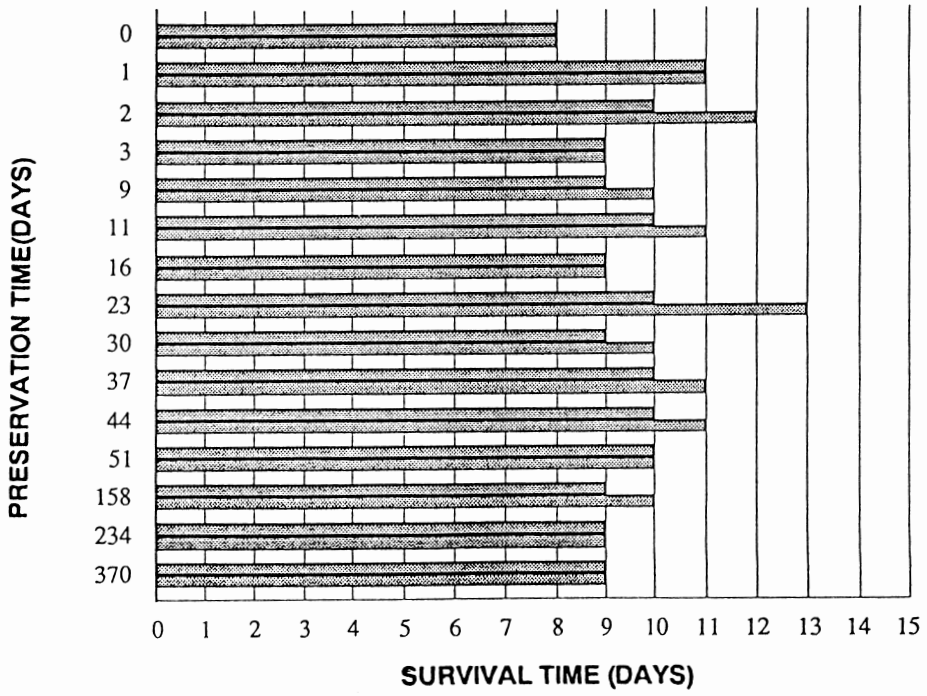

Fig. 2 Survival period of mice inoculated with preserved peritoneal exudate obtained from mice at 42 hr after infection of *Toxoplasma* RH strain.

ing it was observed that 60% (7.25×10^6/ml) of peritoneal cells were infected, and the number of free tachyzoites (1.83×10^7/ml) was twice as much as that of infected peritoneal cells. The materials just before freezing killed animals 5 days after inoculation. The harvests at 3 days preservation and thereafter up to 371 days killed animals after 8, 9 or 10 days of inoculation with an average of 9 days.

Figure 2 shows the survival time of mice inoculated with preserved peritoneal exudate obtained 42 hrs after infection of *Toxoplasma* RH strain. Thirty four percent (2.1×10^7/ml) of peritoneal cells were infected, and the number of free tachyzoites (1.46×10^8/ml) was 7 times as much as that of infected peritoneal cells. In this experiment, it was shown that the material stored for more than 1 year also killed mice after 9–10 days of peritoneal inoculation.

On the other hand, on the 4th day of infection with RH strain, most of the *Toxoplasma* parasites in the ascitic fluid were free tachyzoites (1.2×10^8/ml). When this material was inoculated into mouse peritoneal cavity just before freezing, the animals were killed within 7 days of infection. The peritoneal exudate kept up to 131 days killed some mice due to toxoplasma infection, but the preserved materials kept for 177 and 343 days did not kill any experimental animals (Fig. 3). All the mice that survived were examined for cyst formation in their brains but no cyst was observed by compressed preparation. On the other hand, the serum antibody titer was positive using latex agglutination test.

Peritoneal exudate from infected mice and infected cultured cells have been mainly used for cryopreservation of the parasite. Cell culture itself takes time and needs a lot of work. Thus peritoneal exudate has been largely employed in the previous works. However, in our previous experience, long term storage has not been attained all the time with peritoneal exudate, obtained from mice infected several days previously.

It is known that both free tachyzoites and those parasitised cells exist in the peritoneal exudate, and their proportion differs greatly according to the time after infection. Therefore, peritoneal exudate obtained at 36 and 42 hrs of infection which contained substantial number of parasitized cells, and the materials after 4 days which contained mostly free

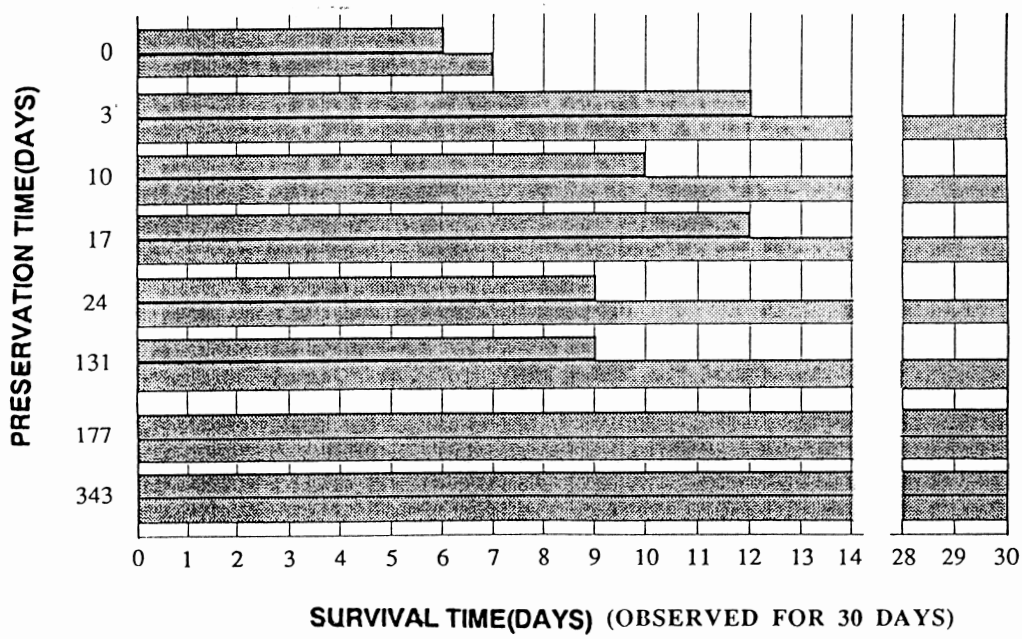

Fig. 3 Survival period of mice inoculated with preserved peritoneal exudate obtained from mice at 4 days after infection of *Toxoplasma* RH strain.

tachyzoites were compared for their suitability to cryopreservation. It is concluded from this experiment that the material including many parasitized cells is the essential factor for long term preservation of *Toxoplasma gondii*.

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