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Rat macrophage activity against *Toxoplasma gondii* studied by electron microscopy

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Abstract: An electrom microscope model was used to study the effect of rat peritoneal macrophages on *Toxoplasma gondii*. 10^7 tachyzoites were inyected i.p. in 30 days-old rats. After 1, 2, 4, 8 and 24 h peritoneal exudate was withdrawn and infected phagocytic cells were prepared for electronic microscope studies. *Toxoplasma* organisms inside of rat macrophages showed remarkable lesions such as vacuolization and organisms were totally lysed inside of macrophages of more than 8 h infection rats. The results confirm at molecular level, the importance of rat macrophages in the natural adaptation of this rodent to *T. gondii*.

MATERIAL AND METHODS

Experimental animals. Thirty-day Sprague-Dowley rats and 30 day-Wistar mice were used in these experiments. All the animals were kept in cages at 21 ± 1°C and fed *ad libitum*.

Parasites. The RH strain of *Toxoplasma gondii* with known characteristics was used to infect the experimental animals.

Infection and collection of macrophages. Groups of 3 rats were inoculated i.p. with 10^7 *Toxoplasma* tachyzoites obtained from 3-4 day infected mice. After 1, 2, 4, 8 and 24 hours, 6 ml of Minimal Essential Medium supplemented with L-glutamine and 20% of fetal calf serum and antibiotics (100 u/ml Penicillin G, Glaxo y 100 ug streptomycin) were injected in the peritoneal cavity and then the exudate was withdrawn. Samples of each group were collected in a cold bath for subsequent process. Mouse peritoneal exudate used as controls was obtained in a similar way, except that only 3 day *Toxoplasma* infected animals were studied.

Studies by methylene blue staining. To determine the tachyzoite lesions with light microscopy, we used the methylene blue staining technic (Endo and Kobayashi, 1976). Rat peritoneal exudates obtained after 15 and 30 min. and 1, 2, 4, 8 hr infection were studied as follows using 3 rats (10^7 tachyzoites per rat) in each case.

Part of the exudate was passed 5 times through a No. 27 gauge needle to release intracellular parasites (lysed). Lysed or not lysed exudate were stained with methylene blue prepared as it is done for the Dye Test (Sabin-Feldman, 1948). The number of stained and non-stained parasites were counted and percentages of both were determined. Non-lysed exudate was used to detect and observe those non-phagocytized organisms.

Electron microscopy. Peritoneal cells were concentrated by centrifugation (100 g for 8 min) and fixed in 2,5% (v/v) glutaraldehyde buffered in 0.1 M fosfate buffer (pH 7.2) for 2 h.

Material was treated with 1% (w/v) O_sO_4 for 1 ha and then washed, dehydrated in graded ethanol, transfered to propylene oxided and embedded in Epoxy (Polyscience Inc.). Ultra thin sections were cut in a Sorvall microtome, stained with uranil acetate (1 h) and lead citrate (1/2 h) and the studied with a Hitachi-H-300 electron microscope.

RESULTS

Light microscopy

Methylene blue staining of peritoneal exudate from infected rats showed that the percentage of lysed parasites (non-stained) was higher as the infection time increased (Fig. 1).

Small vacuoles were observed in the released parasites as early as 1 h after infection. These vacuoles increased in size in the organisms obtained from peritoneal exudate of rats with more infection time until the organisms appeared totally lysed.

Electron microscopy

Tachyzoites found in macrophages of 3 day-infected mice showed the normal cytological aspects previously described (Scholtyseck, 1973). Organisms included in the parasitophorous vacuoles presented a regularly dense cytoplasm without any important lesion or alteration. Nucleus (N) and cytoplasmic membrane (cm) as well as rhoptries and conoid did no present any anormality either (Fig. 2 and 3).

Organisms observed inside of rat macrophages after 1 h infection presented some alterations (Fig. 4, 5 and 6).

Initial parasite degeneration was observed in a tachyzoite inside of the phagocytic vacuole (pv), since small vacuoles (arrows) were found in the cytoplasm (Fig. 4).

Vacuolization was more evident in some tachyzoites (arrows) observed in other macrophages (Fig. 5) and not only cytoplasm lesions but also cellular membrane destruction was clearly shown in some organisms (Fig. 6).

After 2 h infection some intracellular organisms showed a more pronounced vacuolization (Fig. 7 and 8, arrows) and the apical complex of one tachyzoite, apparently was ruptured (Fig. 9 and 10). Some amorphous material was present around the conoid.

Some vacuoles were observed also in the parasite (arrows).

Macrophges obtained from 4 h infected rats presented *Toxoplasma* organisms (arrows) with many vacuoles (Fig. 11) and in some parasites there was an intense degeneration (Fig. 12). Such effect was even more evident in organisms found inside of rat macrophages after 8 h infection (Figs. 13, 14 and 15). Thus, not only the vacuoles in the tachyzoites were more visible (Fig. 13 and 14) but also some of the parasites presented important lesions in the cellular membrane (Fig. 15, arrows).

Macrophages in samples of 24 h infection or more did not present any organisms.

DISCUSSION

Studies carried out *in vitro* showed that the number of *Toxoplasma* tachyzoites inside of rat peritoneal macrophages was lower after 24 hrs infection as compared with the organisms found in macrophages from susceptible animals (Chinchilla *et al.*, 1981 b, 1982). However we do not know whether the low number of parasites is due to killing or just due to multiplication failure. The results obtained in the experiments reported here indicated that destruction of parasites is probably the principal mechanism for the extraordinary resistance of the white rat. In fact a progressive lysis of *Toxoplasma* tachyzoites was seen inside of rat macrophages. Vacuolization started as early as 1 h after infection (Figs. 1, 5, 6), which indicates that the enzymatic effect begins as soon as the parasite invades the macrophage and concludes in a very short period of time. These findings correlate with previous work where *Toxoplasma* was not seen in the rat peritoneal exudate after 1 day infection (Chinchilla *et al.*, 1981a).

The effect seems to be vert strong since after 8 h there is important membrane destruction besides the vacuole formation (Figs. 13, 14, 15) and in the methylene blue test it was very difficult to find any intact organisms (Fig. 1).

Since some of the vacuoles were found surrounding the nucleous (Fig. 8) it is possible that the effect starts along the endoplasmic reticulum as reported by Mehlhorn *et al.* (1984) for the effect of Triazinones on several stages of *Eimeria*. *Toxoplasma* tachyzoites were clearly included in a parasitophorous vacuole (Figs. 8, 9, 10) since the characteristic tubules (Nichols and O'Connors, 1981) could be seen (arrows). The parasite observed in Figs. 9 and 10 presented an alteration in the anterior part that cannot be interpreted as the effect of rhoptries secretion since it appears during host-cell invasion (Nichols *et al.*, 1983). Thus we think that those photographs (Figs. 9, 10) show another type of lesion in the parasite.

Killing of the organisms cannot be related to antibody-complement effect since in our experiments lysis does not start with swelling of inner membrane as at has been demonstrated in electron microscopy studies of *Toxoplasma* treated for the Sabin-Feldman dye test (Endo and Kokayashi, 1976).

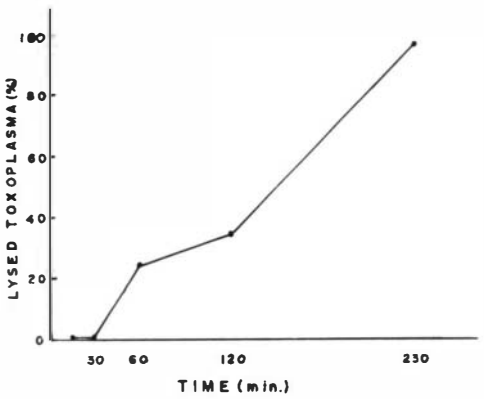


Fig. 1. Lysis of *Toxoplasma* inside of rat peritoneal macrophages.

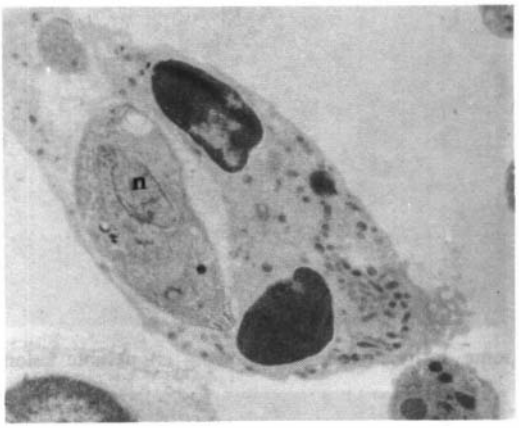


Fig. 2. *Toxoplasma* tachyzoite inside of a macrophage from a 3 days-infected mouse.

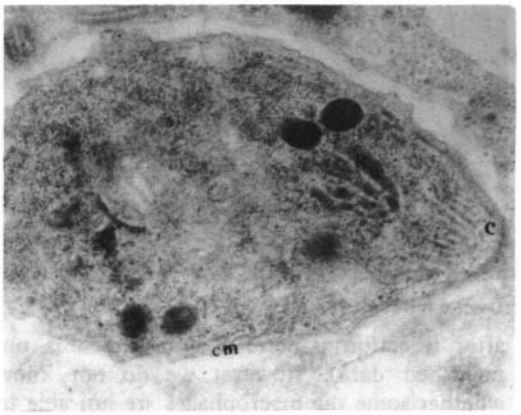


Fig. 3. A detail of one *Toxoplasma* tachyzoite. Note the intact conoide (c). rhoptries (r) and the rest of the organism.

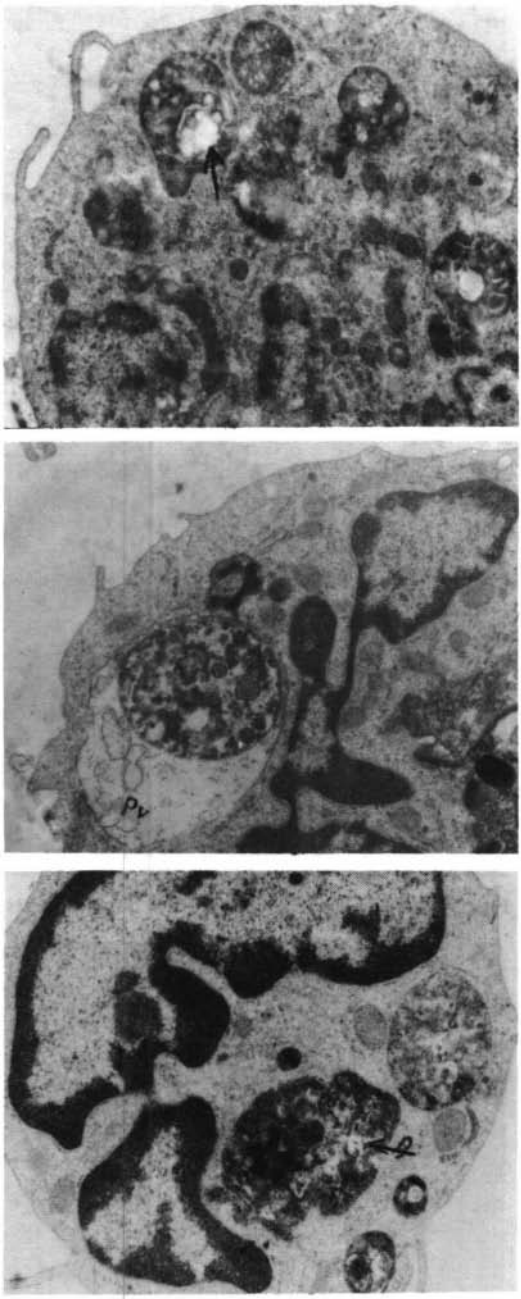
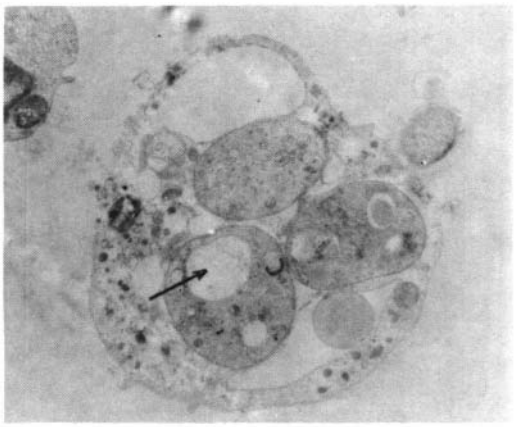
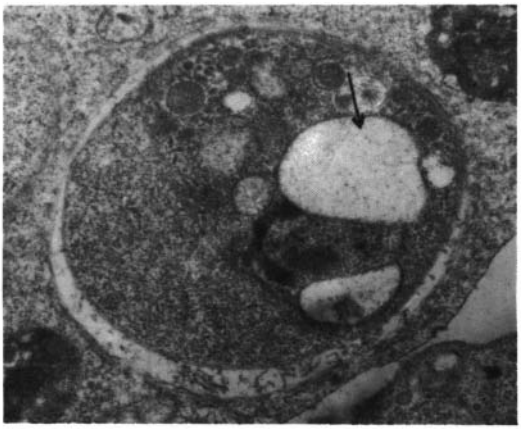
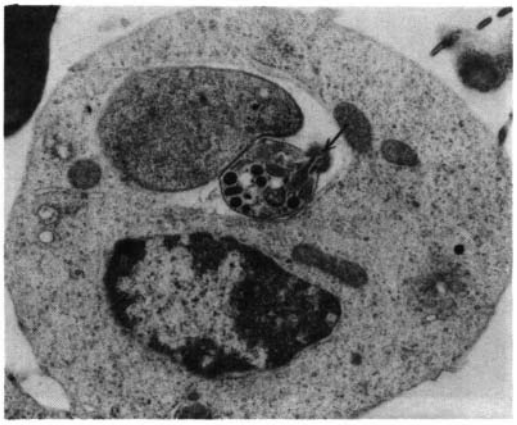


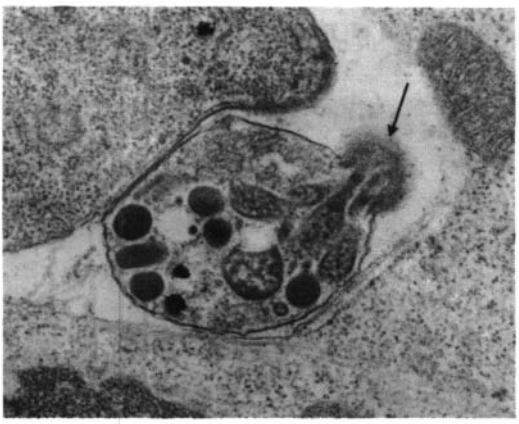
Fig. 4, 5, 6. *Toxoplasma* organisms in macrophages from 1 h infected rats.

Also, vacuolization is quite different in both cases. For all these reasons we think that the destruction of parasites observed here is due to a strong intracellular effect of peritoneal macrophages which represent the first barrier against infection in the white rat. This effect could be similar to the halide myeloperoxidase system

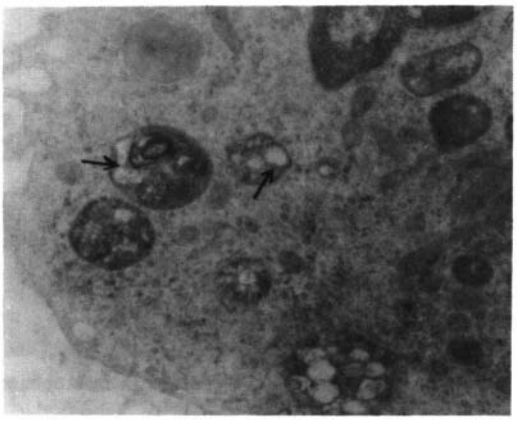


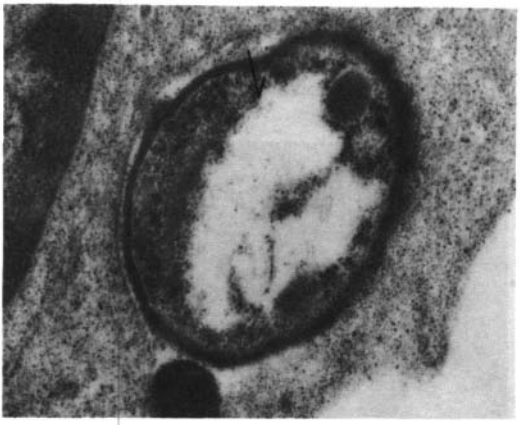






Figs. 7, 8, 9, 10. Macrophages from 2 h infected rats with some tachyzoites inside showing citoplasmic lesions.

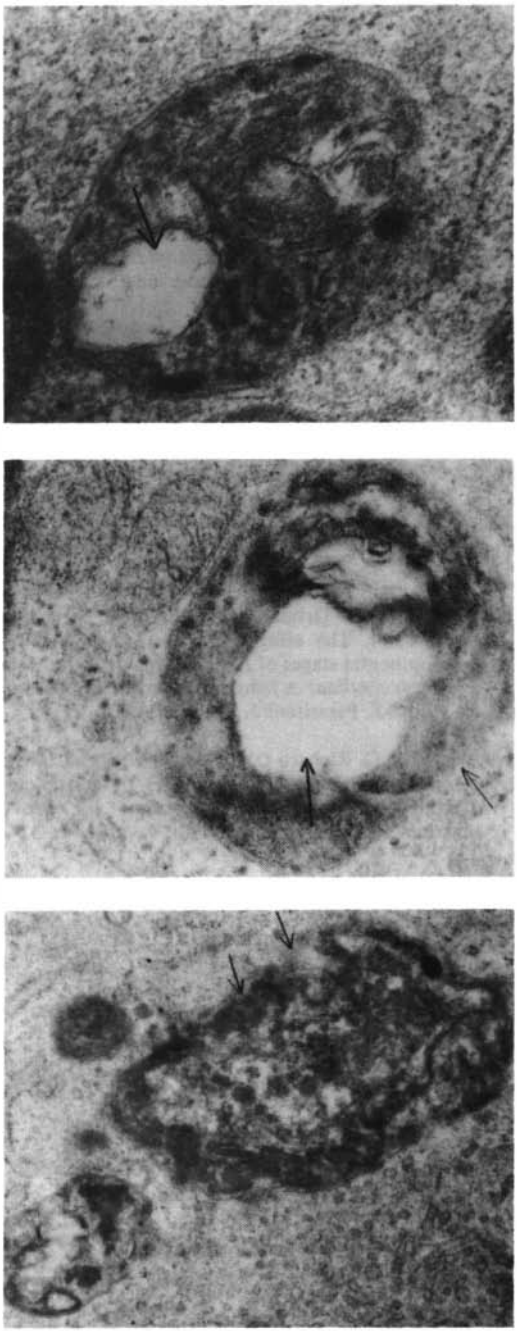




Figs. 11, 12. Very lysed tachyzoites inside of macro phages from 4 h infected rats.

(Klebanoff, 1968) or a oxigen independent system (Cline *et al.*, 1978).

We have found that some of the organisms escape the lethal effect in the peritoneal cavity of the rat since *Toxoplasma* cysts have been found in brains, lung and other organs 30 days after infection (Guerrero and Chinchilla unpublished data). However we do not know whether some the macrophages are not able to kill *Toxoplasma* or if this issoporoid can be transported by other cells such as any type of granulocytes. At any rate, once the tachyzoites



Figs. 13, 14 15. *Toxoplasma* organisms in macrophages from 8 h infected rats showing the intense lysis of the tachyzoites.

reach some organs such as lung and others, it is probable that they can survive and multiply to some extent since, as we have demonstrated (Chinchilla *et al.*, 1981b, 1982) this parasite can multiply easily in alveolar macrophages.

RESUMEN

Se hizo un estudio al microscopio electrónico del efecto de los macrófagos de la rata blanca sobre el *Toxoplasma gondii*. Ratas blancas de un mes fueron inoculados i.p. con 10^7 taquizoitos y después de 1, 2, 4, 8 y 24 horas se les extrajo el exudado periotoneal el cual fue procesado para el estudio al microscopio electrónico de las células fagocíticas infectadas.

Los toxoplasmas observados dentro de los macrófagos de ratas mostraron lesiones muy evidentes tales como formación de vacuolas y destrucción de la membrana celular. Este efecto fue aumentando conforme pasaba el tiempo de infección y ya después de 8 horas todos los organismos estaban lisados.

Estos resultados confirman a nivel molecular, la importancia de los macrófagos en la adaptación natural de la rata blanca al *T. gondii*.

Some animals are resistent to *Toxoplasma* infection. The resistance of rats, for example, has been studied by several authors (See references in Chinchilla *et al.*, 1981a). We have shown remarkable natural adaptation by these animals since they are able to resist 10^7 LD_{50}-*Toxoplasma* without any symptomatic manifestation. Among the factors inducing this kind of resistance, we found that age is very important since 1 to 5 day-old rats were less resistant than 10, 15 or 30 day-old animals (Chinchilla *et al.*, 1981a). In addition, macrophages seem to be another important factor. In fact, peritoneal macrophages from normal rats presented a very low number of *Toxoplasma* tachyzoites after 24 hours infection, as compared with mice, guinea pig or hamster macrophages where huge numbers of parasites were found. This finding was confirmed *in vivo* and *in vitro* (Chinchilla *et al.*, 1981b, 1982). Furthermore in experiments following the fate of *Toxoplasma* in peritoneal exudate, we have shown that 3 days after the infection in the rat it is almost impossible to recover any parasites, even by means of mice inoculation of that exudate (Guerrero and Chinchilla, unpublished data). On the basis of all this evidence it appears that rat macrophages have some internal destructive effect against *Toxoplasma* tachyzoites. In this paper we report the results of some electron microscope studies intended to demonstrate the phenomena at the molecular level.

ACKNOWLEDGEMENTS

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