# FATE OF Toxoplasma gondii IN MACROPHAGES in vitro DEPENDING ON VIRULENCE OF THE PARASITES

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A comparative light-optical and submicroscopic study was made of the development of strains of Toxoplasma gondii with high and low virulence in macrophages in vitro. By contrast with the active multiplication of the highly virulent strain, most toxoplasmas of the less virulent strain underwent disintegration within a few hours after penetrating into the cell, so that as a result, 24-48 h after infection, they were completely digested. Submicroscopic investigation revealed no significant morphological changes in the macrophages infected by the less virulent toxoplasmas, whereas the nuclei of macrophages infected with highly virulent toxoplasma were considerably altered. Disintegration of the parasites within the phagosome is accompanied by gradual transformation, initially of the double or triple membrane of the cyst containing the parasite into a single membrane and disappearance of the accessory layer from the mitochondria and structures of the endoplasmic reticulum surrounding the membrane of a cyst.

KEY WORDS: Toxoplasma; virulence, macrophages.

Cellular and humoral factors have been shown to play an important role in immunity to toxoplasmosis. However, the role of macrophages in the development of both primary infection and of reinfection by toxoplasmas still remains largely unexplained. Since toxoplasmas are obligate intracellular parasites, and since they spend part of their life cycle in cells of the mononuclear phagocytic system (the RES), especially macrophages, one possible approach to the study of the problem of immunity in toxoplasmosis is to study interaction between the parasite and macrophages in vitro [4, 5, 8, 9].

This paper is supplementary to the first report [3] and deals with the comparative study of interaction bet en the macrophage host cell and toxoplasmas of strains with different virulence.

## EXPERIMENTAL METHOD

Strains of peritoneal macrophages from various animals (albino mice, guinea pigs, rats), infected with proliferative forms of toxoplasmas of the highly virulent RH strain and of the Lagrave* strain with low virulence, by the method described previously [3], were used as the experimental model. Cultures of macrophages grown for 48 h were infected with toxoplasmas, fixed at various times during development of the infection, and stained by the Romanovsky–Giemsa method as described previously [3]. Material for submicroscopic study was fixed in 1% glutaraldehyde, postfixed in osmium tetroxide, dehydrated, and embedded in Araldite in the usual way [6]. Multiplication of the toxoplasmas in the macrophages was assessed quantitatively by counting the "relative number of infective units" (RNIU) by the method of Lycke and Lund [7].

*The strain was isolated in France from the placenta of a woman who had given birth to a child with congenital toxoplasmosis; it was kindly made available by Professor Desmond (Paris), to whom the writers are grateful.

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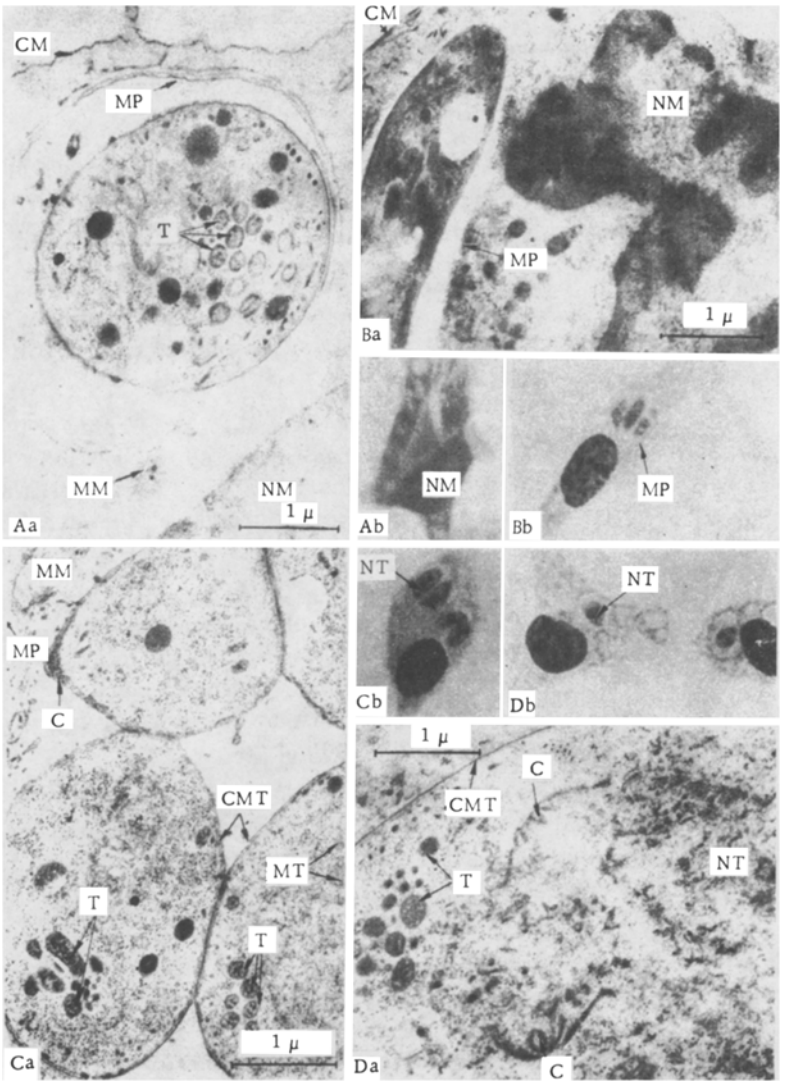


Fig. 1. Interaction between toxoplasmas of the low-virulence Lagrave strain with mouse macrophages in vitro. Electron (a) and light (b) microscopy. A) Toxoplasma inside a phagosome 3 h after infection, B) degeneration of toxoplasma inside phagosome after 24 h, C) formation of two daughter toxoplasmas in phagosome after 24 h, D) endodyogeny: beginning of formation of cenoids of two daughter cells. CM) Cytoplasmic membrane of macrophage, NM) nucleus of macrophage, MM) mitochondrion of macrophage, MP) membrane of phagosome, CMT) cytoplasmic membrane of toxoplasma, T) toxoneme, MT) mitochondrion of toxoplasma, NT) nucleus of toxoplasma, C) conoid.

## EXPERIMENTAL RESULTS

The results of the study of interaction between macrophages of different origin and toxoplasmas of the low-virulence Lagrave strain and the virulent RH strain by light and electron microscopy were as follows. During the first hour of contact between macrophages and toxoplasmas no significant difference was observed in phagocytosis and intracellular penetration of the toxoplasmas depending on the strain used. By 2-3 h after infection most of the parasites were inside vacuoles in the macrophages surrounded by a double or triple membrane (Fig. 1: Aa and Ab). However, from 3-4 h after infection many intracellular toxoplasmas of the low-virulence strain had disintegrated (Fig. 1: Ba and Bb); there was hardly any difference in the intensity of digestion of the parasites in cells from animals that differed in susceptibility (macrophages of mice and rats; Fig. 2). The toxoplasmas became longer and crescent-shaped, with highly condensed regions

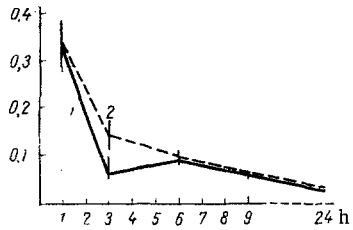


Fig. 2. Results of counting RNIU during development of infection in macrophages: 1) albino mice, 2) rats. Abscissa, time after infection (in h); ordinate, RNIU.

of strongly vacuolated cytoplasm. Around the single membrane bounding the clone of toxoplasmas the accessory layer was missing from the mitochondria and elements of the endoplasmic reticulum (Fig. 1: Ba), though it was present where the parasites were multiplying (Fig. 1: Aa).

At the same times, in a few macrophages obtained either from susceptible or from resistant animals, multiplication of several toxoplasmas of the Lagrave strain was observed in vacuoles surrounded by a clear single membrane (Fig. 1: Ba and Bb). However, in all the cultures at this time most of the toxoplasmas of this strain had completed only one division by endodyogeny (Fig. 1: Da and Db), in contrast to the intensive proliferation of the virulent strain.

The cytoplasm of most infected cells was vacuolated and contained remnants of digested toxoplasmas. Submicroscopic study of macrophage cultures from mice infected with toxoplasmas of the Lagrave strain revealed no significant morphological changes in the cells, whereas the nuclei of macrophages infected with the virulent RH strain were considerably altered.

Most of the intracellular parasites 1-2 days after infection by toxoplasmas of the low-virulence strain appeared to be digested, as shown by the absence of stained parasites under the light microscope and by the abundance of pictures of parasite degeneration in the electron microscope. Toxoplasmas of the low-virulence strain had no marked cytopathogenic action on cultures of macrophages. The formation of cystic structures of toxoplasmas characteristic of this particular low-virulence Lagrave strain when grown, for example, in a culture of chick fibroblasts, likewise could not be observed.

As yet little is known of the true role of macrophages in pathology caused by protozoans. The most interesting results have been obtained with respect to the protective function of macrophages during their infection by the parasites Plasmodium berghei, Trypanosoma cruzi, Leishmania donovani, and Toxoplasma gondii, some of which spend part of their life cycle in macrophages.

Work which has begun on the study of relations between toxoplasmas of a virulent strain and macrophages in vitro at the submicroscopic level [5] has revealed the complex character of these relations even within a population of the same strain. This confirms the results obtained by the present writers when studying interaction between toxoplasmas of the virulent RH strain and macrophages of different origin [1-3], when a short period of disintegration, evidently of the weaker population of parasites, was observed, followed by active proliferation of the other parts of the population with a clearly defined cytopathogenic action on the macrophages, culminating in their death.

The present investigation shows that toxoplasmas of the low-virulence strain undergo disintegration within a few hours of their penetration into the cytoplasm of the cells, and by 24-48 h most of the intracellular toxoplasmas are digested. Under these circumstances the monolayer of macrophages remains intact, whereas after infection of the cultures by toxoplasmas of the virulent RH strain the cells begin to break up and to separate from the surface of the glass as early as 24 h after infection (the concentration of parasites introduced in both cases was the same).

The fate of the toxoplasmas phagocytosed by the macrophages, it is assumed, may depend on various factors and under the conditions used it is largely determined by the virulence of the strain. Toxoplasmas of the low-virulence Lagrave strain are distinguished by a slow rate of reproduction and the interval between two generations differs considerably from that for the virulent strain even if grown in cultures of fibroblasts (5-6 h for the RH strain and 12-14 h for the Lagrave strain). The specific functional properties of the macrophages must also be taken into consideration. Nevertheless, even at this stage the ability of toxoplasmas to multiply in a culture of macrophages can evidently serve as an additional criterion for the description of strains as well as the criterion of virulence toward albino mice, which is widely used at the present time.

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