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SYNTHESIS OF PYRIMIDIC BASE BY INTRACELLULAR FORMS OF *TOXOPLASMA GONDII* IN CELL CULTURE

Sakae YONEDA (1), Renato Piza de Souza CARVALHO (2) and Beatriz Ribas CASTELLANI (3)

SUMMARY

The Authors have studied the pyrimidine metabolism of intracellular forms of *Toxoplasma gondii* in cell culture. The parasites have exhibited a large incorporation of uridine and an almost negligible uptake of orotic acid. These data suggest that the intracellular form of *T. gondii* in cell culture has its pyrimidic compounds built up preferentially through a "salvation" pathway.

INTRODUCTION

In the last two decades, a fairly large number of papers have been published on various aspects of *Toxoplasma gondii*. EYLES & COLEMAN [2]; FRENKEL & HITCHINGS [5]; SILVA [18]; EBRINGER et al. [1]; HSU [8], to name but a few, have carried out extensive investigations on the action of certain drugs on this parasite, a research field to which we now offer some experimental contribution.

According to LYCKE & LUND [15], in tissue culture, the extracellular forms of *T. gondii* apparently utilize p-aminobenzoic acid, folic and folinic acids for coenzyme synthesis, in a biochemical pattern suggestive of the coexistence of more than a single metabolic pathway, e.g.: sulphonamide interferes with the intracellular synthesis of folic acid and pyrimethamine, as well as in the folic acid conversion to folinic acid. As shown by GREENBERG & JAENICKE [6], this acid acts particularly on the synthesis of the formil donor coenzyme, which is especially important for the synthesis of purine nucleotides.

In this contribution to the biological knowledge of *T. gondii*, we have tried to work out some further metabolic aspects of the parasite's pyrimidine synthesis.

MATERIAL AND METHODS

The sediment obtained by centrifugation at 2,500 r.p.m. for 15 minutes, of peritoneal washings with saline from mice previously infected with *T. gondii* (*), Rh strain, was submitted to two additional washings in Hanks-Wallace solution [7] and inoculated into HeLa [12] or Sirc [13] cell cultures, and incubated at 37°C. The nutrient medium employed was Eagle's minimal medium, with 2% inactivated fetal calf serum. On the second day, the cells were heavily infected. After one washing in Hanks-Wallace solution, fresh nutrient medium containing tritiated uridine (**) or tritiated orotic acid (***) both

(*) Rh strains, *T. gondii* was kindly supplied by Dr. Saburo Hyakutake from Divisão de Biologia Médica, Instituto Adolfo Lutz, São Paulo, Brasil.

(**) H^3-Uridine, specific activity 25.6 c/mM, kindly supplied by Prof. Antonio Sesso from Depto. de Histologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil.

(***) 5-H^3-Orotic acid, specific activity 14.1 c/Mml Schwarz Bioresearch Inc. Orangeburg, New York, kindly supplied by Dr. Maria Mitzi Brentani and Prof. Ricardo Renzo Brentani from Laboratório de Oncologia Experimental, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brasil.

(1) Departamento de Histologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil
(2) Instituto de Medicina Tropical de São Paulo e Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil.
(3) Instituto de Educação Domingos Faustino Sarmento, São Paulo, Brasil.

to a final concentration of 0.5 μc/ml, was added only to the experimental tubes.

Radioactive labelling was allowed to occur for twelve to twenty hours of incubation at 37°C. The nutrient medium was then discarded, the cell monolayers were washed with balanced salt solution, and subsequently treated with a 0.1% trypsin solution during 30 minutes at 37°C, for cell dissociation and rupture.

Smears of the washed culture sediments were fixed in Bouin's solution for 10 minutes and submitted to routine histological processing, before radioautography after MESSIER & LEBLOND [16] and KOPRIVA & LEBLOND [10] with K^5 nuclear emulsion (Ilford, England). Exposure time varyied from 45 to 78 days; the preparations were finally counterstained with Giemsa. For each experimental group and control cultures as well, a minimum of ten slides were prepared.

RESULTS

The radioautographic preparations correspondent to the H^3-orotic acid treated group, show scarce and irregular distribution of silver granules (Fig. 1), while the other experimental group, assaying the incorporation of H^3-uridine by *T. gondii*, exhibits a remarkable and regular distribution pattern of the silver granules (Fig. 2).

Control cultures are consistently negative (Fig. 3).

Fig. 1 — Cell culture forms of *Toxoplasma gondii*. H^3-orotic acid uptake; scarce and irregular distribution of silver granules. Giemsa stain, 1,160 X.
Fig. 2 — Cell culture forms of *Toxoplasma gondii*. H^3-uridine uptake; heavy incorporation denoted by abundant silver granules on the parasites. Giemsa stain, 1,160 X.
Fig. 3 — Cell culture forms of *Toxoplasma gondii*. Control group; no silver granules present. Giemsa stain, 1,160 X.

DISCUSSION

Our results point to a close similarity between the *T. gondii* metabolic pathway for pyrimidine synthesis, and those of *Trypanosoma cruzi* in its every biological stage, as reported by KIMURA & FERNANDES [9], and by YONEDA [21]. The incorporation of uridine proceeds at a speedy rate, whereas the uptake of orotic acid is rather meaningless.

According to WRIGHT et al. [19] and LIEBERMAN et al. [14], orotic acid is a precursor of pyrimidic base nucleotides; according to KORNBERG [11], it is an intermediary product in the "de novo" biosynthesis, while uracil is an intermediary in the "salvation" pathway.

Thus, it can be inferred that the intracellular (merozoite) stage of *T. gondii*, exhibiting heavy uridine uptake and low incorporation of orotic acid, develops the synthesis of its pyrimidic base nucleotides mainly through a "salvation" pathway.

The data so far obtained by us do not allow any further conclusions with regard to the particular pyrimidine metabolic patterns followed by each evolutionary stage of *T. gondii* as already established for *T. cruzi* (KIMURA & FERNANDES [9]; YONEDA [21]). In the latter organism, it is known that, concerning

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purines, the preferential biosynthetic pathway varies with each morphological stage (FERNANDES & CASTELLANI [4]; REY & FERNANDES [17]; and YONEDA [20]). In tissue culture, the amastigote forms (leishmania) exhibit the "de novo" biosynthesis of purine as preferential pathway, in opposition to the epimastigote (crithidiae) and trypomastigote (trypanosome) stages, which develop a "salvation" pattern for the synthesis of its purines (YONEDA [20]). On the other hand, FERNANDES & CASTELLANI [4] have demonstrated that the "salvation" pattern prevails for the different evolutive stages grown in artificial media.

It stands out as most unusual, the observation that both organisms, *T. cruzi* and *T. gondii*, displaying intense multiplying activity while thriving intracellularly, should resort almost exclusively to a "salvation" pathway for the synthesis of its pyrimidic base nucleotides, in opposition to the patterns followed in the make-up of its purinic base nucleotides.

Recalling on basis of LYCKE & LUND's [15] experimental results on the different response of the intra- and extracellular forms of *T. gondii* to experimental drug therapy, we might extrapolate to this sporozoan a biochemical behaviour similar to the *T. cruzi*.

RESUMO

Síntese da base pirimidínica por formas intracelulares do Toxoplasma gondii *em cultura de células*

Os Autores estudaram o metabolismo pirimídico das formas intracelulares do *Toxoplasma gondii* em cultivo de células.

Os parasitos mostraram grande incorporação de uridina e quase nula de ácido orótico. Estes dados sugerem que as formas intracelulares (merozóito) do *T. gondii* em cultivo de células têm a via de biosíntese de seus compostos pirimídicos preferentemente por via de "salvação".

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