

PHAGOCYTOSIS OF *Leishmania mexicana* L. BY FSDC CELL LINE

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The Langerhans cell (LC) represents the dendritic cell system in the epidermis and in other stratified epithelia(1). The LC captures, processes, and presents antigens to the T cells to generate a specific immune response. Phagocytosis of *Leishmania* parasites in amastigote form was previously demonstrated (2) by parasites incubation with isolated LC of mice epidermis; thus 20% of LCs were parasitized. However, using the cell line FSDDC, phagocytosis was observed containing both promastigotes (the parasite form skin inoculated by the vector insect) and amastigotes (the parasite form adopted within its mammal host); thus 7% of the host cells contained promastigotes and 36% of them contained amastigotes (3).

This work describes the fine structure of *Leishmania mexicana* (promastigotes and amastigotes) phagocytosis events by the cell line FSDC. This cell line was obtained in the University of Milan from dendritic cells of mice fetal epidermis immortalized by the retroviral transduction of the v-mvc oncogen(4). The promastigotes was obtained from tissue culture (24°C) of epidermal lesion from Balb/c infected mice 30 days after inoculation with the *Leishmania mexicana* WR-L11 strain. The amastigotes was directly obtained from animal lesions. The cellular line was co-cultured with the parasites in a 5:1 proportion using IMDM media (37°C and 5% of CO₂ during 24 hours). Control and infected cultures were fixed with buffered 3% glutaraldehyde 30 min and post-fixed with 1% OsO₄ 1h. The samples were then dehydrated in ethanol and embedded in Epon-Araldite mixture.

Phagocytosis count were made on semithin sections (1µm) stained with toluidine blue. Thin sections (60 nm) were stained with uranyl acetate and lead citrate and then observed in a Zeiss EM109 electron microscope. FSDC cells phagocytosing promastigotes (13%) and amastigotes (20%) were counted. The percentages of FSDC cells counted phagocytosing parasites were the following: 8% contained a single parasite (amastigote or promastigote); 12% of FSDC cells phagocytosed 2 or more amastigotes and 5% of the cells contained 2 or more promastigotes. At electron microscope promastigotes penetrating FSDC cells by the flagellum or the posterior pole were observed. Parasitophore vacuoles (PV) containing promastigotes were very narrow, therefore the space between the parasite and the PV membrane was only of 100-200nm and only one or two parasites for each PV were observed. On the contrary PV containing amastigotes were bigger (8 µm diameter). Each PV enclosed one or several parasites both free or attached to the PV membrane by the posterior pole. In conclusion differences in phagocytosis count of each parasite form and PV features were observed. Some of these differences studied in macrophages have been related with intrinsic factors in each parasite form.

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References

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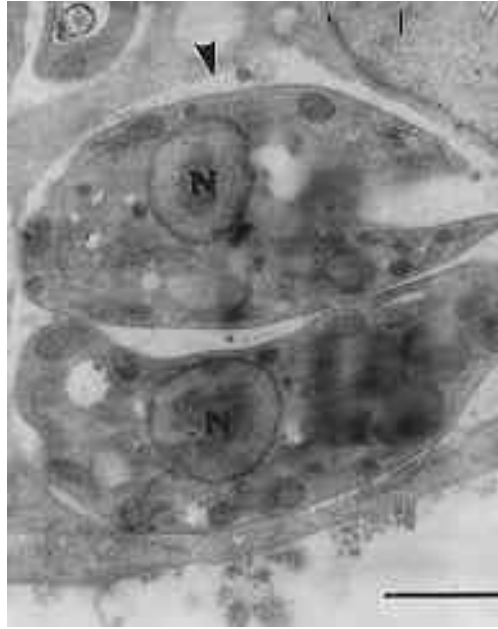


Figure 1. Promastigotes within a parasitophorous vacuole of a FSDC. Phagosomal membrane (arrows). Nucleus= N. Barr= 1 μ m

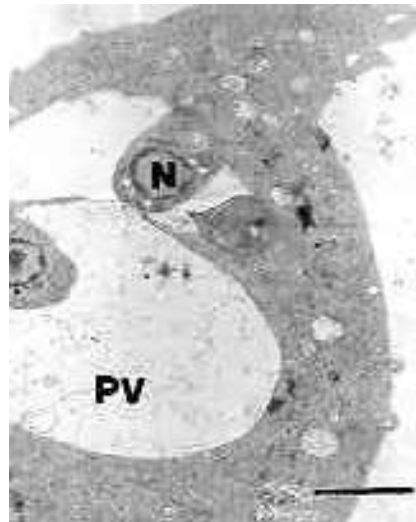


Figure 2. Amastigote inside a large PV of FSDC. Amastigote nucleus= N. Barr= 3 μ m