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Cell-to-cell transfer of *Leishmania* amastigotes observed by multidimensional live imaging: participation of extrusomes in host cell egress and reinfection

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The last step of Leishmania intracellular life cycle is the egress of amastigotes from the host cell and their uptake by adjacent cells. Taking advantage of multidimensional live imaging of macrophage cultures infected with L. amazonensis, we describe some previously unseen features of this process [1,2].

Amastigotes were transferred from cell to cell when the donor host macrophage collapses; transfer between live cells was not detected. We found that amastigotes were extruded from the collapsed host macrophage within zeiotic structures (blebs) rich in late endosome/lysosome components such as LAMP1 and Rab7. These structures were classified in this study as parasitophorous extrusomes. The extrusome was selectively internalized by vicinal macrophages and the rescued amastigotes, carrying host lysosomal components attached to their surfaces (but dissociated from host cytoskeleton components such as actin) were able to survive in recipient macrophages (Figure 1). To induce cell-to-cell amastigote transfer, the nuclei of infected GFP-expressing macrophages were microirradiated by 405 nm laser, what induced apoptosis in these cells but not in vicinal macrophages [3] (Figure 2). Non-irradiated macrophages were able to rescue amastigotes from apoptotic, irradiated cells, suggesting that amastigotes benefit from host cell apoptosis to spread among other cells. Amastigote transfer occurred concomitantly to macrophage GFP leakage, what is suggestive of pore formation on host cell membrane during the process. Transfer was also stimulated when macrophages are treated with streptolysin O, a pore-forming protein innocuous to amastigotes.

The participation of host lysosomal components associated with amastigote surfaces in transfer of parasites was investigated; amastigotes isolated from LAMP1/LAMP2 knockout cells were less phagocytosed by macrophage cultures when compared to amastigotes isolated from wild-type cells. Amastigotes covered with LAMP proteins also increased the production of TGF-β by macrophages in comparison with amastigotes displaying host membrane caps devoid of these lysosomal components.

We provide evidence that amastigotes, enclosed within host cell membranes, can be transferred from cell to cell without full exposure to the extracellular milieu. The presence of lysosomal components on egressing amastigotes increase their uptake by vicinal cells and modulate cytokine production, what represents an important strategy developed by parasite to evade host immune system[1].

1. N. Friedrich, M. Hagedorn, D. Soldati-Favre and T. Soldati, Microbiol Mol Biol Rev 76 (2012), p. 707-720.
2. M. G. Rittig and C. Bogdan, Parasitol Today 16 (2000), p. 292-297.
3. L. Soustelle, B. Aigouy, M. L. Asensio and A. Giangrande, Neural Dev 3 (2008), p. 11.

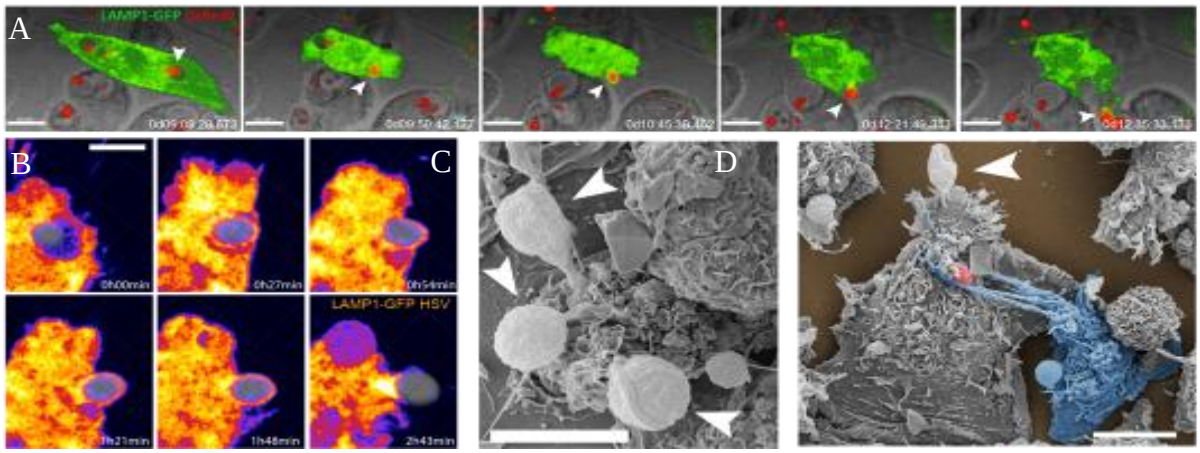

Figure 1. Amastigotes are transferred from cell to cell associated to phagolysosomal components. (A) Multidimensional imaging of RAW 264.7 cells expressing GFP-tagged Lysosome-associated membrane protein 1 (LAMP1, in green) and infected with L. amazonensis-DsRed2 (red) for 24 hours. RAW cells were microirradiated (405 nm near UV laser, 400 Hz, 120 seconds) to induce extrusion and transference of amastigotes. Arrowheads indicate an extruded amastigote rescued from vicinal macrophage. The amastigote carries a polarized cap of LAMP1 protein. Time of image acquisition is represented by days:hours:minutes:seconds:miliseconds (d:hh:mm:ss:sss). Image acquisition started after 24 hours of infection plus 2 minutes of microirradiation. Bars=10 µm. (B) LAMP1 is expressed on amastigote extrusion. The temporal sequence of the extrusion event presented in A shows that amastigote is surrounded by phagolysosomal components during the host cell egress. Relative time of extrusion is represented by hours:minutes (h:mm). (C-D) Field-emission scanning electron microscopies of BMDMØ infected with L. amazonensis-DsRed2 for 20 days. The left image (C) shows a shrunk macrophage presenting extrusions with dimensions compatible to amastigotes (arrowheads). Bar=5 µm. On the right image (D), one macrophage (colored in blue) interacts with an oval-shaped structure (red) from another macrophage. This structure has similar dimensions to an amastigote. Bar=10 µm.

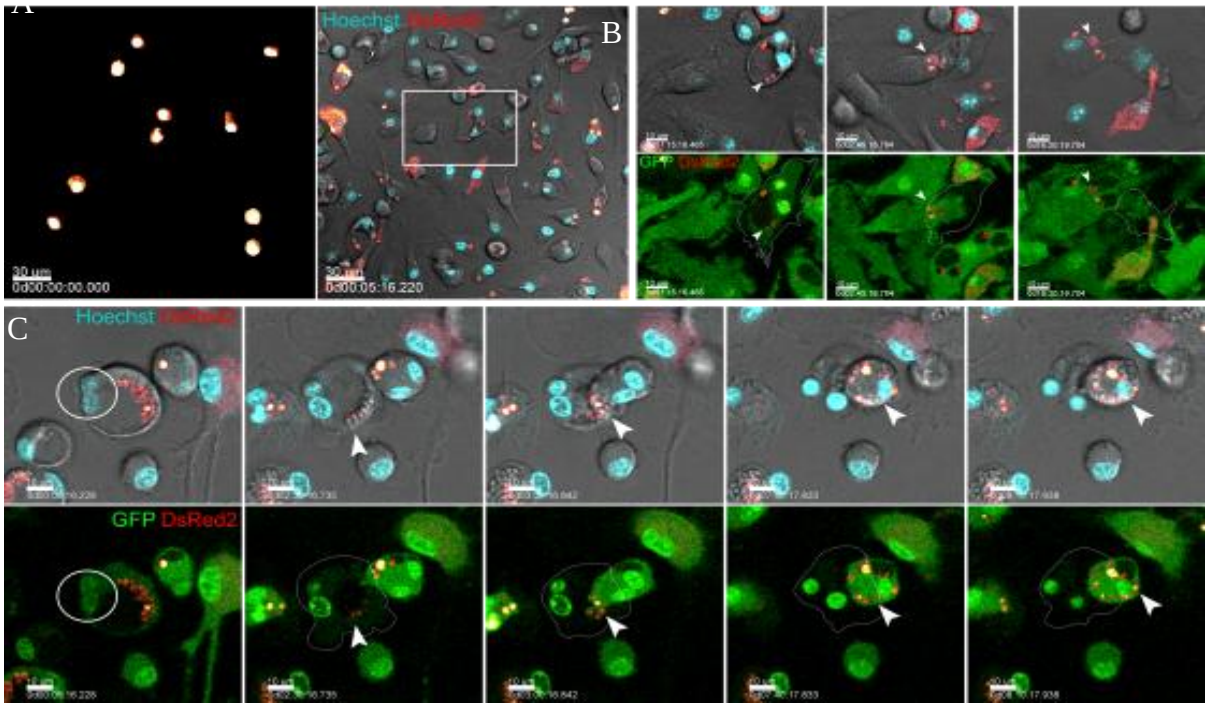

Figure 2. Microirradiation of host cell nuclei induces cell death and amastigote transfer. (A) On the left panel, microscopic field (63x objective) in which 10 nuclei were microirradiated with near UV (405 nm) laser at 400Hz for 300 seconds. On the right panel, the corresponding field showing microirradiated BMDMØ-GFP infected for 72-120 hours (*L. amazonensis*-DsRed2 in red and Hoechst 33342 in cyan, over DIC image). Square indicates an example of an amastigote transfer induced by microirradiation shown in B. Bar=30 µm. (B-C) Live cell imaging of amastigote transfer induced by microirradiation. The upper panel shows *L. amazonensis*-DsRed2 in red and Hoechst 33342 in cyan merged with DIC image at defined time intervals. The lower panel shows the corresponding green channel (GFP fluorescence) and *L. amazonensis*-DsRed2 (in red). Amastigotes are transferred with concomitant GFP leak out of the host cell (arrowhead). Time of image acquisition is represented by days:hours:minutes:seconds:miliseconds (d:hh:mm:ss:sss). Image acquisition started after 72 hours (B) or 120 hours (C) of infection plus 5 minutes of microirradiation. Bar=10 µm.