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LETTER TO THE EDITOR

LOOSE AND COMPACT AGGLOMERATES OF 50 NM MICROVESICLES DERIVED FROM GOLGI AND ENDOPLASMIC RETICULUM MEMBRANES IN PRE- AND IN -APOPTOTIC MYCOPLASMA INFECTED HELA CELLS: HOST-PARASITE INTERACTIONS UNDER THE TRANSMISSION ELECTRON MICROSCOPE

São Paulo, November 17, 2014

Dear Editor

The fine structure of apoptotic HeLa cells from cultures contaminated with mycoplasma in early and in advanced stages of the cell demise process differs from those so far described in apoptotic cells. The observed changes are enhanced after exposure of the cells to staurosporine. At low microscopic magnifications cells that have apparent normal cytoplasm and nuclei, actually may be harbouring cystic-like profile(s) of parasitic origin in an altered cytoplasm. The membranes of the transitional elements of the endoplasmic reticulum (TER) appear fragmented in irregular branching stripes of the smooth component of the TER (Fig. 1, white asterisks in L delimited area). The concentration of the rough endoplasmic reticulum (RER) membranes is less than in normal HeLa cells. Near to the smooth ER tubule-saccular elements lie groups of 50 nm microvesicles aside stacked, thin, various sized profiles of Golgi saccules ( ]). The 50 nm microvesicles bud off mainly from the periphery of the stacked Golgi elements (Fig 1 thin arrow heads inside line U) and also from the extremities of smooth ER tubules (Fig. 1 small arrows). Small groups of compacted microvesicles are noted in cells still maintaining normal nuclear appearance (not shown). With the start of chromatin condensation progressively larger compact microvesicular clusters are formed. These attain sizes larger (Fig. 2) than those of the clusters of microvesicles derived from the fragmentation of Golgi saccules seen in mitotic (LUCOCQ *et al.,* 1989; SESSO *et al.,* 1999) and in apoptotic (SESSO *et al.,* 1999) cells. Contemporaneously two major cytoplasmic alterations may be noted in contaminated cells namely when treated with staurosporine. Occasionally, both deformations appear in the same cell. One, is progressive cytoplasmic loss by formation at the cell periphery of blebs that separate from the inner cytoplasm (Figs. 3 and 4) or by localized detachment of sectors of the peripheral cytoplasm with various forms and sizes (not shown). In some cells, the remainder thin, cytoplasm with few mitochondria and rough ER profiles surrounds the nucleus in a ring-like form. Such small cells are noted in heavily contaminated samples. Some of the cells exhibit sectors of the cytoplasm with a reticulated appearance. Such net-like regions are composed by various sized tubular and ellipsoidal, apparently empty profiles. It is unclear if the smooth membranes that compose these regions with reticulated aspect, may have derived from the Golgi apparatus. The shape and size of the empty spaces correspond to those from villus-like formations seen free close to and emerging from the cell surface. In contaminated cells namely after staurosporine treatment the free villus - like forms are seen sprouting from the cell surface and also free nearby. Spheroidal 50-100nm (thin arrow in Fig. 5) profiles with inner structure identical to that of villus-like elements are consistently proximate to the fake villi. Vestiges of what can be remnants of the villus-simile structures and/or of the parasite itself are seen in these spaces (Figs. 6 and 7).

It is yet undetermined whether the early structural changes expressed by foci of assembled microvesicles at the transitional endoplasmic reticulum-Golgi interface is an exclusive type of membranal alteration preceding overt apoptosis in mycoplasma infected cells.

All cytoplasmic membrane bound organelles as peroxisomes, lysosome-endosomes and the Golgi apparatus derive from microvesicles that bud off from the ER. The ER is also mobilized by promoters of cellular stress (references in DOLAI & ADAK, 2014). The here described structural deviation of the ER-Golgi interface from the normal condition may represent more than only a mycoplasma induced alteration of the programmed cell death mechanism. It is speculative, whether this initial accumulation of microvesicles in cells with typical normal nuclei is part of a general forewarning mechanism of cell defence. In a less intense cell stress than that occurring here the observed changes of the TER could eventually pass undetected under the transmission electron microscope.

The mycoplasmas fine structures of our samples are identical (NIR-PAZ *et al.,* 2002; KORNSPAN *et al.,* 2010;) and similar (EDWARDS & FOGH, 1960; HUMMELER *et al.,* 1965; TAYLOR-ROBINSON *et al.,* 1991) to those from various mycoplasmas strains seen in cultures and in infected cells.

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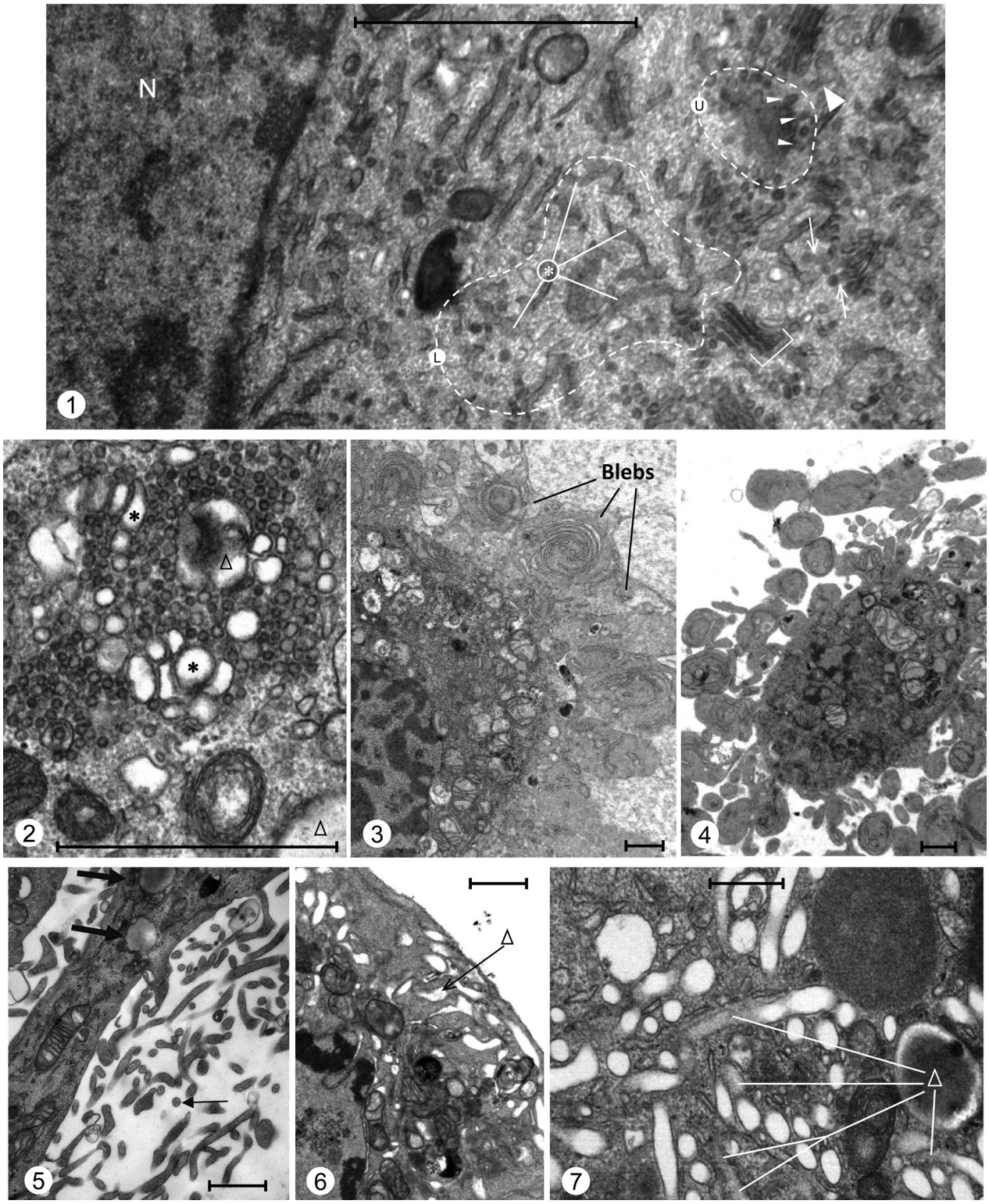

Figs. 1-7 are a synopsis of fine structural observations in some 50 samples of staurosporine (0.1 μM - 2.0 μM for 2 - 24h) and 10 control samples of HeLa cell cultures contaminated with mycoplasma. Fig. 1 is from an apparent normal cell at low microscopic exam. Figs 2, 6 and 7 and 3 and 4 are from apoptotic cells exposed to staurosporine 0.5 μM/3h and 0.1 μM/6h, respectively. Fig. 5 is from a non apoptotic cell exposed to staurosporine 0.1 μM/4h. Bars -1 μM. **Fig. 1 -** HeLa cell-from a heavily contaminated cell culture devoid of staurosporine where apoptosis occurred. Normal nucleus (N). Smooth, branched ER profiles (extremities of thin white lines departing from the central white asterisks in the area delimited by the lower (L) dotted line. Conglomerates of 50 nm (lower and upper right part of the figure). Part of the microvesicles bud off from smooth ER membranes (arrow head) and from tubular elements (small arrows). The majority of

the grouped microvesicles pinch off from the extremities of stacked Golgi saccules (thin arrow heads at the periphery of the frontally exposed dense ellipsoidal-like profile delimited by U)]. Stacked, thin Golgi saccular profiles of various lengths that often appear dense (**]**). **Fig. 2 -** Large, compact conglomeration of various sized microvesicles with predominance of the ones with 50 nm. Groups of former dilated stacked Golgi saccules (black asterisks) are interspersed among the microvesicles. A large such dilated saccule contains remnants of a parasite or of material of parasitic origin (MPO) (empty triangle). **Fig. 3 -** The cytoplasm of this apoptotic cell is delimited in two concentric major regions. The peripheral one is partitioned into adjacent blebs containing predominantly membranes of the ER. The inner part of the cytoplasm contains clustered, swollen mitochondria. **Fig. 4 -** Dismantling of the peripheral apoptotic cytoplasm by a contemporaneous detachment of the previously formed bleb regions. **Fig. 5 -** Non apoptotic infected (cysts of parasitic origin, upper arrows) cell, with an abnormally elongated thin cytoplasm. The free villus-like structures often appear curved aside spheroidal 50-100 nm (thin arrow) elements. They assemble in various degrees in the sectors of the contaminated cells apoptotic or not undergoing progressive dismantling of the peripheral cytoplasm. **Fig. 6 -** Most of the cytoplasm above the apoptotic nucleus is occupied by membrane bound void spaces of various sizes and forms. The mitochondria are unusually dense, a common occurrence in contaminated cells, apoptotic or not. Adherent to the limiting membrane of the space indicated (open triangle) a fluffy material possibly derived from MPO. **Fig. 7 -** Sector of an apoptotic cytoplasm with net-like membranal arrangement as in Fig. 6. Compact mass of parasitic material in a cystic-like form (open triangle). The upper, middle and vertical white lines indicate remnants of parasitic origin that were not completely removed by the processing of the cells. Part of an elongate membrane bound space possibly still fully occupied by MPO is indicated by the lower line that branches in two.

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