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Characterization of *Mycobacterium bovis* Phagosome

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Mycobacterium tuberculosis complex are among the most successful pathogens. Their success resides in the ability to interfere with intracellular traffic avoiding natural pathways of the phagosome maturation [1]. Recently, mycobacteria escape from the phagosome to the cytosol was investigated as an alternative survival strategy. In this context we decided to determine the exact intracellular location of *Mycobacterium bovis* in different host macrophages and characterize the pathogen intracellular niche for survival. The main goal here was to characterize live vs dead *M. bovis spp* phagosome in different host macrophages. Macrophages infection, fluorescence and EM procedures were carried out as described previously [2-4].

Although it is accepted that intracellular mycobacteria reside inside the phagosome, a few studies showed that they are able to escape and persist in the cytosol [5-7]. Our data is in agreement with the persistence inside a phagosome (Fig.1(a)). The lack of acidification is a clear indicator of phagosome maturation arrest. The data presented in table.1 shows that live BCG and *M. bovis* can arrest phagosome acidification, thus being independently of the host used. In contrast phagosome containing heat-killed mycobacteria fully matures within 3 days.

When we evaluated phagolysosome biogenesis following either the fusion with colloidal gold particles (targeted to lysosomes) or the acquisition of the v-ATPase (responsible for pH drop within the phagosome lumen) (Fig.1(b)) the same conclusion is achieved. Figure 1(c) shows that live and heat-killed BCG resides in different compartments in J774 cells. While less than 20 % of phagosomes containing live BCG co-localize with late lysosome markers (LAMP-1, LYAAT and LBPA) the phagosome containing heat-killed BCG acquired most of these markers and much faster. The rate of EEA1 acquisition also supports this assumption. All together our data clearly show that live mycobacteria persist within an immature membrane limited compartment.

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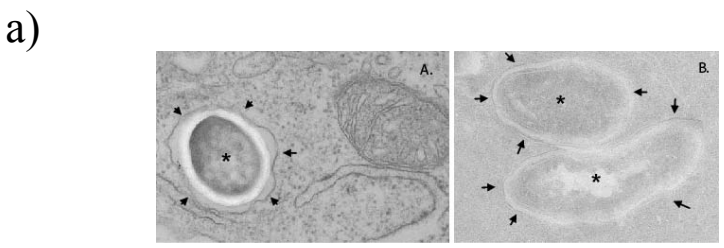
TABLE 1. Percentage of acidified phagosomes containing of live / heat killed *M. bovis spp* in different host macrophages.

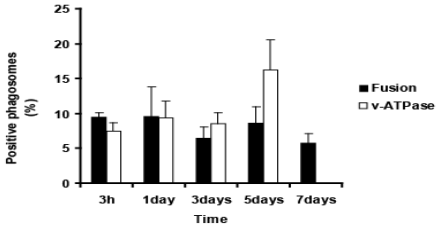
A. Live mycobacteria

	BCG							*M. bovis*					
Time / Cell	3h Average	SD	1day Average	SD	3 days Average	SD	Time / Cell	3h Average	SD	1day Average	SD	3 days Average	SD
J774	13.0	5.0	20.9	6.3	11.5	4.0	J774	10.0	0.4	9.40	2.0	4.40	0.5
Raw	22.0	1.3	24.2	5.2	39.5	6.3	Raw	26.3	3.8	36.1	4.1	33.1	5.0
THP-1	34.4	6.5	42.3	7.5	42.7	3.9	THP-1	53.9	9.9	60.8	16	52.5	9.2
BMM	23.6	0.5	18.1	3.8	20.6	5.5	BMM	---	---	---	---	----	---
HMDM	21.2	6.9	9.00	5.8	9.0	3.6	HMDM	10.1	0.4	9.40	2.0	4.40	0.3
BMDM	21.5	4.3	28.4	7.3	32.5	7.4	BMDM	11.9	0.9	16.4	1.6	15.6	2.6

B. Heat killed mycobacteria

	BCG							*M. bovis*					
Time / Cell	3h Average	SD	1day Average	SD	3 days Average	SD	Time / Cell	3h Average	SD	1day Average	SD	3 days Average	SD
J774	25.4	4.5	75.0	6.9	80.0	5.8	J774	19.4	5.6	30.3	7.5	71.5	13.3
THP-1	64.6	5.6	59.5	4.5	73.0	2.4	THP-1	61.5	1.5	68.8	12.7	67.7	5.4
BMDM	21.9	9.5	82.5	8.6	92.4	3.7	BMDM	19.1	7.4	44.8	13.0	69.0	6.6





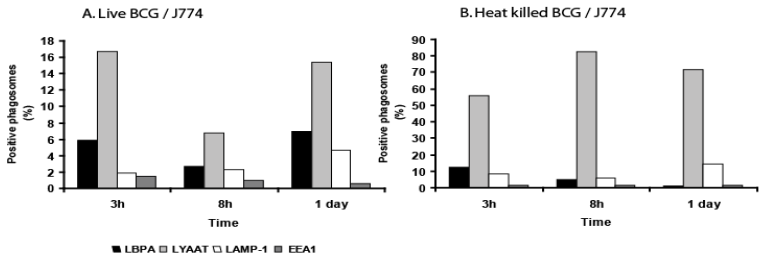


Figure 1. (a) *M. bovis spp* phagosome. Epoxy resin plastic section of J774 cells infected with *M. bovis* (A.) or BCG (B.). Bacteria are marked with (*), arrows indicate the phagosome membrane. (b) Live BCG containing phagosomes fusion with late endosomes /lysosomes in J774 cells. (c) Acquisition of early and late endosome markers by phagosomes containing live or heat killed BCG.

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