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Phosphoinositide involvement in *Leishmania donovani* phagocytosis by Raw 264,7 macrophage

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Introduction:

Phosphoinositides (PIs) are **key regulatory molecules** in cellular functions including signal transduction, membrane trafficking, and cytoskeleton dynamics. Temporal and spatial changes in specific phosphoinositide levels, signal actin rearrangements that support **phagocytosis** and regulate membrane fusion and fission events resulting in **phagosome maturation** (Fig.1).
A number of **obligatory intracellular bacteria**, such as *Salmonella sp*, *Listeria sp.* and *Mycobacterium sp* are known to **modify phosphoinositide metabolism** and/or their signaling cascades as part of their survival mechanism in their host cells (Fig.2) . This has not been addressed thus far for **protozoan parasites.**

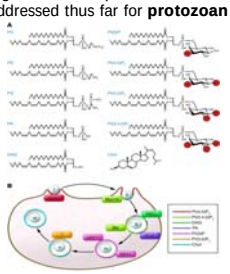

Fig. 1: The involvement of PIs at the different stages of phagocytosis. PI binding proteins known to regulate membrane and cytoskeleton dynamics during phagocytosis are also shown in same colors as PIs. (*Benjamin E. 2008. J Clin Invest.118(6): 2002–2011)*

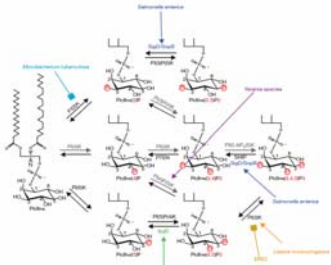

Fig. 2: Main steps in phosphoinositide synthesis and degradation. Steps subverted by bacterial pathogens are shown. (*Pizarro-Cerda 2004 NAT. CELL BIOL.6 (11): 1026-1033)*

Methodology- Results :

Our approach consists of visualizing the localization of specific cytoskeleton or endosomal markers and PIs by **confocal fluorescence microscopy** in fixed cells during the first **3 hours** of *Leishmania* phagocytosis. In these studies we use:

- transiently or stably transfected **RAW264.7** cells expressing GFP or YFP fusions of **Rab5** and **Rab7** proteins and certain PI-binding domains, i.e **PH** from FAPP1, PLCδ1 and Btk proteins, and **PX** from NADPH oxidase subunit p40(^{phox}) and
- transgenic red or green fluorescent *L. donovani* parasites [***L.donovani*-mRFP1** (Poster no 146)**, *L.donovani*-GFP**] (**Fig 4**).

Experiments with IgG opsonised inert particles (1-2 μm size) are performed in parallel to detect differences in the temporal or spatial distribution of the specific PIs that would indicate a subversion of PI metabolism by *Leishmania* during its phagocytic uptake by macrophages.

1. Localization of cytoskeleton and endosomal markers on *Leishmania* or inert bead harboring phagosomes, at specific time points during phagocytosis

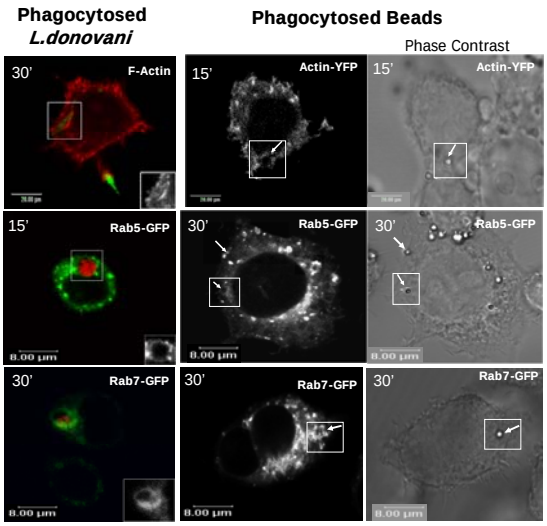

Fig. 5: Confocal Microscopy images of Raw264.7 macrophages phagocytozing *Leishmania* or inert beads (size 1μm). On top left panel F-actin is labeled with phalloidin Alexa546. The time points of phagocytosis are indicated at the top left of each panel. Arrows point to beads.

Conclusions- Prespectives:

PIs and enzymes involved in their metabolism are known to be modulated by intracellular bacterial virulence factors.This aspect remains still poorly explored for protozoan parasites mainly due to the lack of pharmacological inhibitors with specific irreversible effect that act selectively on enzymes of the host cell. Our research is on going. Preliminary results encourage our research hypothesis that *Leishmania* parasites modulate PI metabolism in order to survive and proliferate in the mammalian phagocytes. Our goal is to confirm our initial microscopy observations in fixed cells by *in vivo* imaging studies and biochemical analysis of phosphoinositide levels during *Leishmania* infection, and explore the molecular mechanisms involved in this event as it has been already done for intracellular bacteria pathogens.

***Leishmania sp.* are protozoan parasites** responsible for the wide spectrum of diseases of Leishmaniasis. The life cycle of the parasite includes a non-intracellular stage (promastigote phase) in the insect host and an obligatory intracellular stage (amastigote phase) in phagocytes of the mammalian host where they proiliferate and establish a replicative niche. *Leishmania donovani* is the causal agent of the potentially fatal disease, visceral leishmaniasis (VS), in humans.

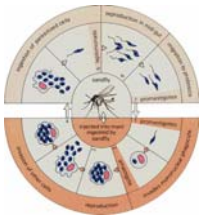

Fig. 3: Life cycle of *Leishmania sp.*

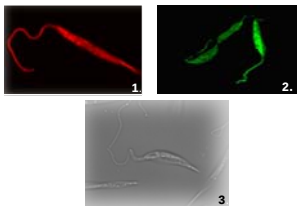

Fig. 4: Leishmania donovani LG13-mRFP1 (**1**), LG13-GFP (**2**) and LG13 wt (**3**) parasites grown in cell culture

Aim of the work:

To investigate the possibility that *Leishmania* promastigotes subvert directly or indirectly the metabolism of the PIs at the initial stage of the infection and during the maturation of the parasitophorous phagosome.

2. Localization of specific PIs on *Leishmania* or bead harboring phagosomes at specific time points during phagocytosis.

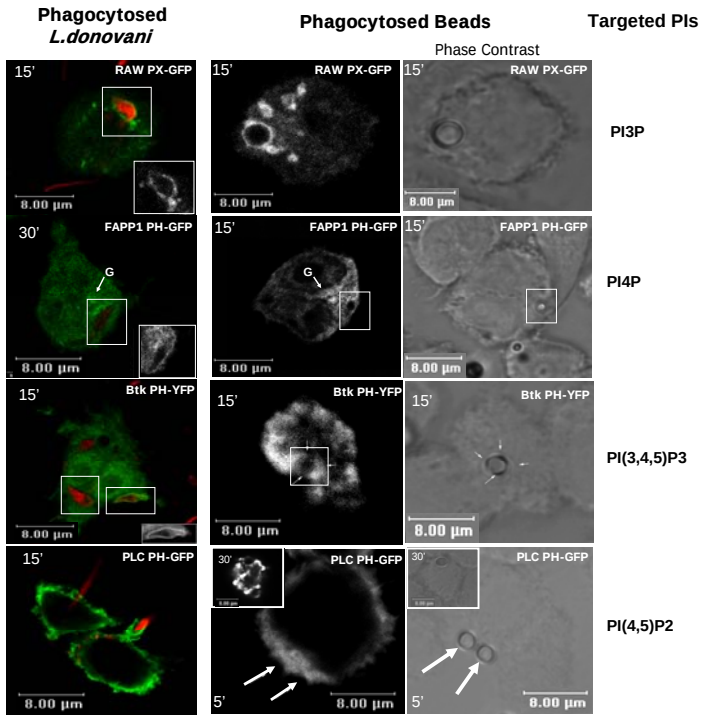

Fig. 6 Phagocytosis of *L.donovani-mRFP1* or inert beads (size 1-2μm) by Raw264.7 macrophages expressing YFP or GFP fusions of PBDs followed by confocal microscopy. **Left top**: Time points of phagocytosis. **Right top**: YFP or GFP fusions of PBDs. **G** : Golgi. Arrows point to beads.

3. Quantification of actin and PI3P distribution during the first 15 min to 3 hrs of phagocytosis

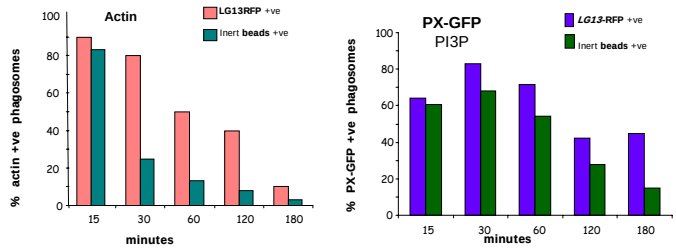

Fig. 7: Phagocytosis of *L.donovani-mRFP1* or inert beads was followed by confocal microscopy in fixed cells. A number of 80-100 *Leishmania* or bead harboring phagosomes were counted for each time point. Actin or PX-GFP +ve phagosomes were enumerated. Results were expressed as % of the total no of phagosomes +ve for the markers.
Results indicate that PI3P and Actin seem to stay much longer on the parasitophorous phagosomes.