- BMP 5764 -

"VII - Amastigotas"

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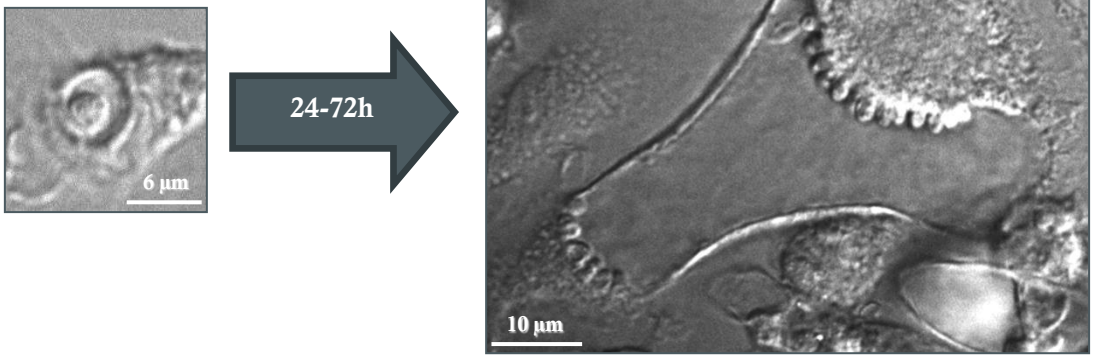
Setembro | 2020

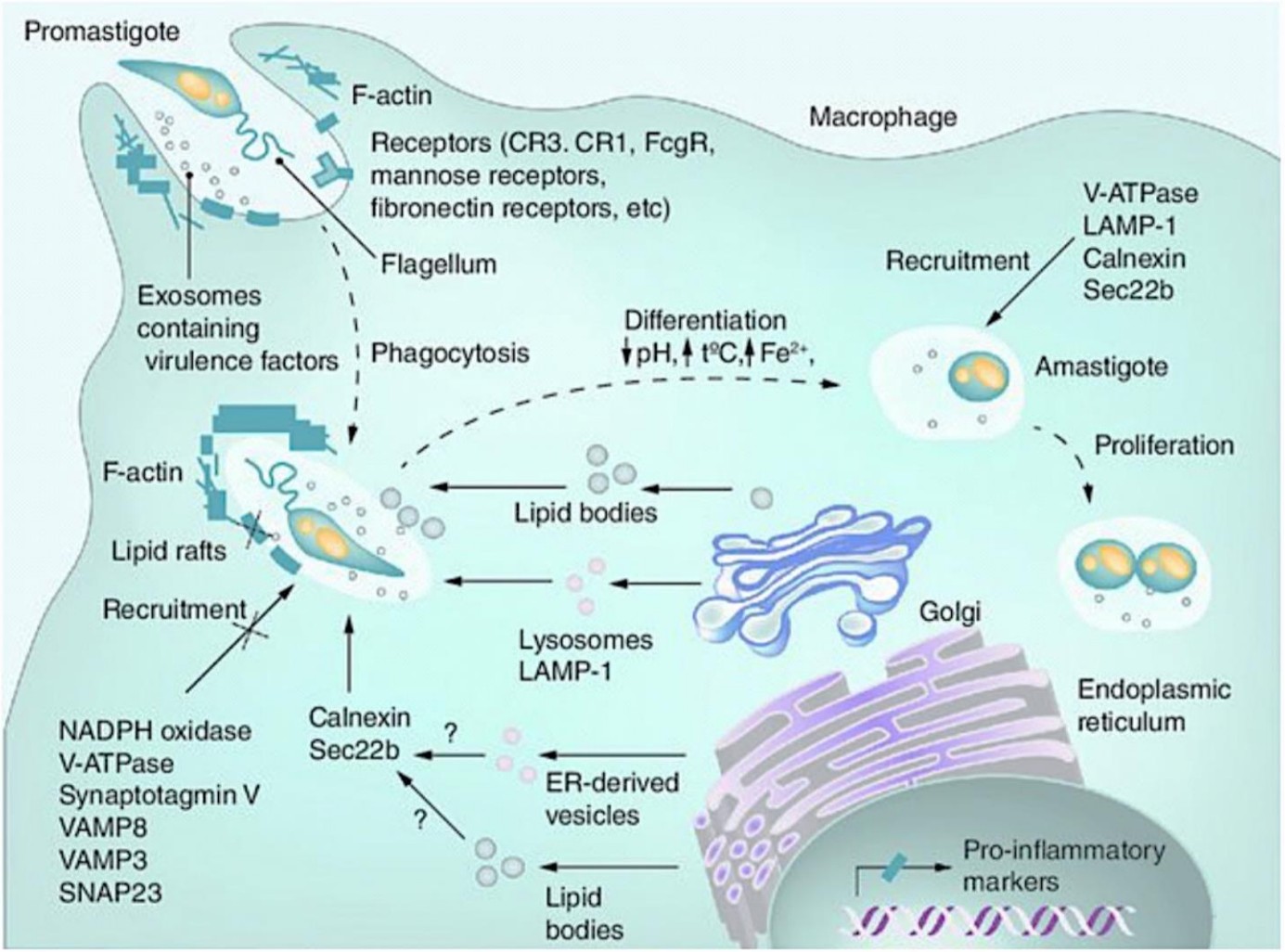
UNICAMP


6 µm
24-72h
10 µm

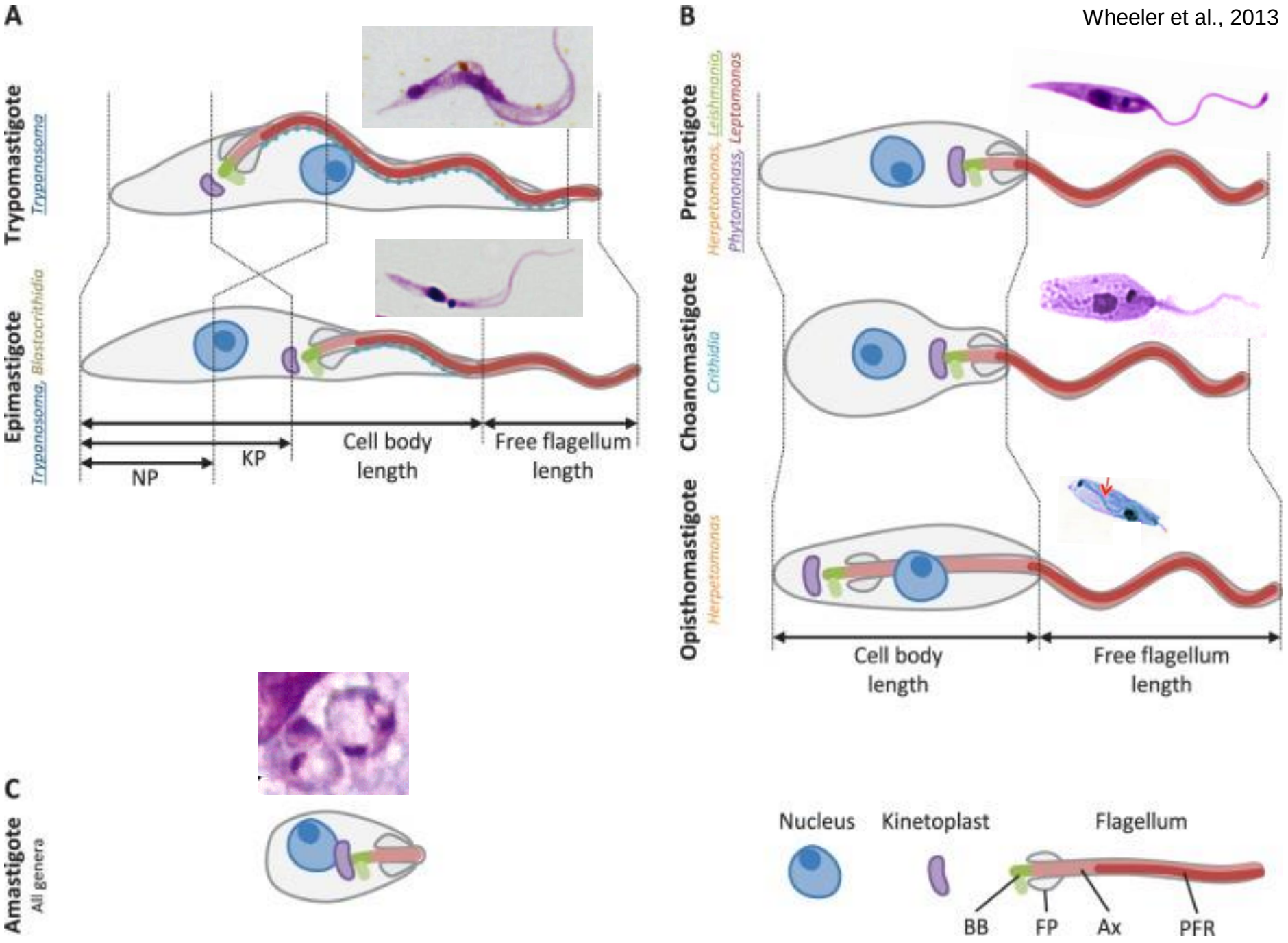
Future Microbiol. 2015;10(1):111-29. 10.2217/fmb.14.103.

Leishmania and the macrophage: a multifaceted interaction

Maria Podinovskaia, Albert Descoteaux

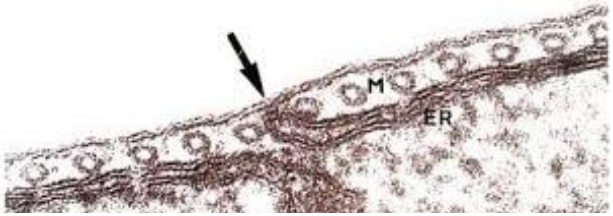


Wheeler et al., 2013

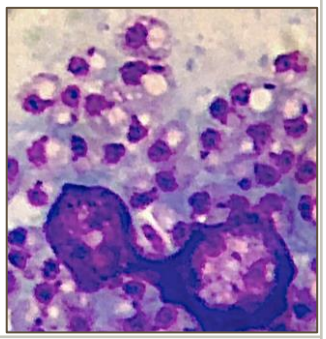


- Amastigotes lack the paraxial rod and have larger flagellar pocket
- The subpellicullar microtubules end subterminally
- Electron dense and membranous vesicles are highly evident in the flagellar pocket area of the amastigotes; the electron dense material was considered to derive from the megasomal/electron dense organelles that could be found abutting the flagellar pocket
- Presence of megasomes, the large lysosomes seen in some *Leishmania* spp, cysteine proteinases, and sensitivity to L -leucine-methyl ester (LeuOMe)
- Relevant differences in the secretory/endocytic pathway!!!

These large amastigote lysosomes (up to 15% of the cell volume) correlate with high levels of developmentally regulated cysteine proteinase
(Duboise et al. 1994; Brooks et al. 2000; Ueda-Nakamura et al. 2002)

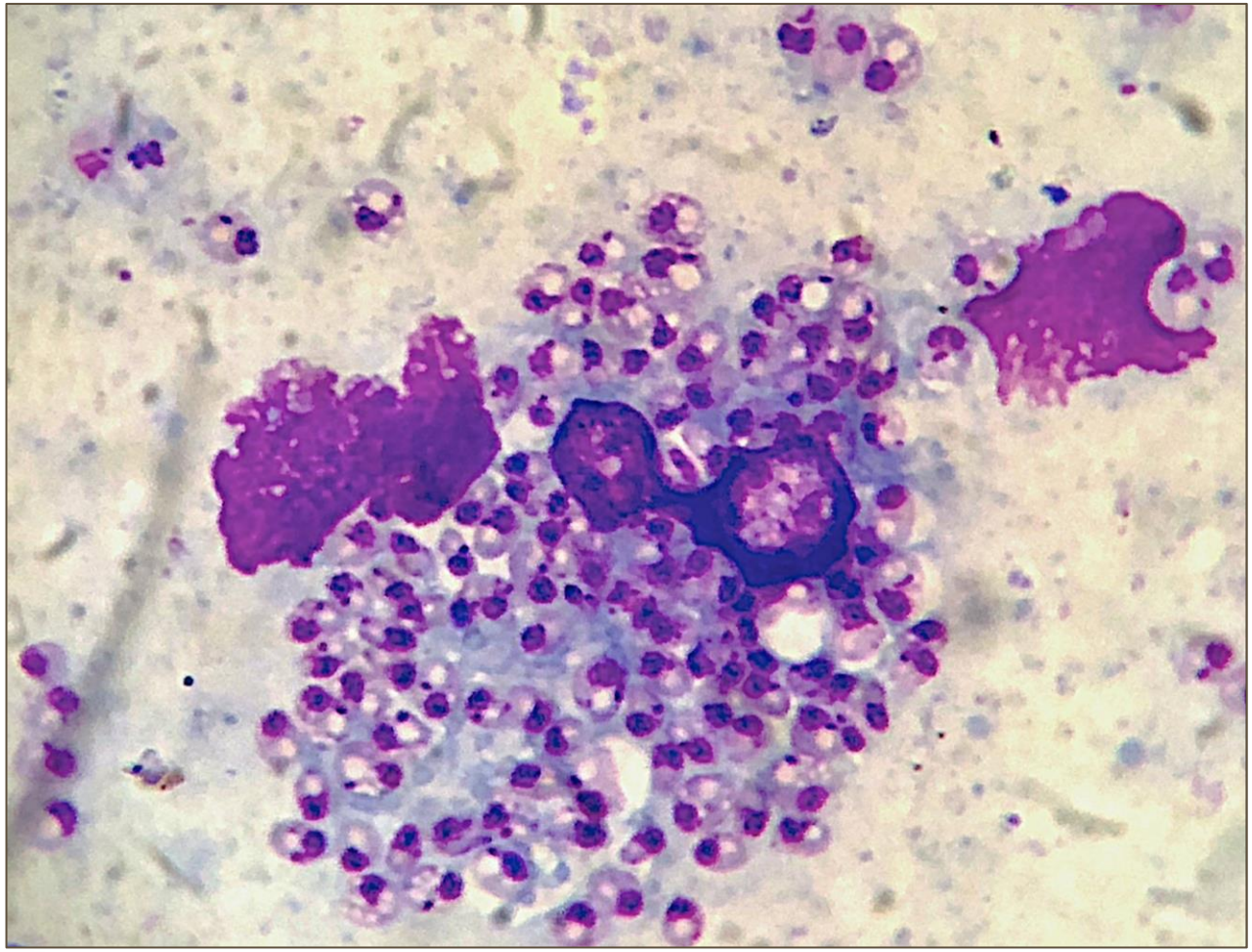


Cross-section of subpellicular microtubules of *L.amazonensis.* The arrow points to a profile of the endoplasmic reticulum eventually inserting between the regularly spaced microtubules (Pimenta and De Souza 1985)



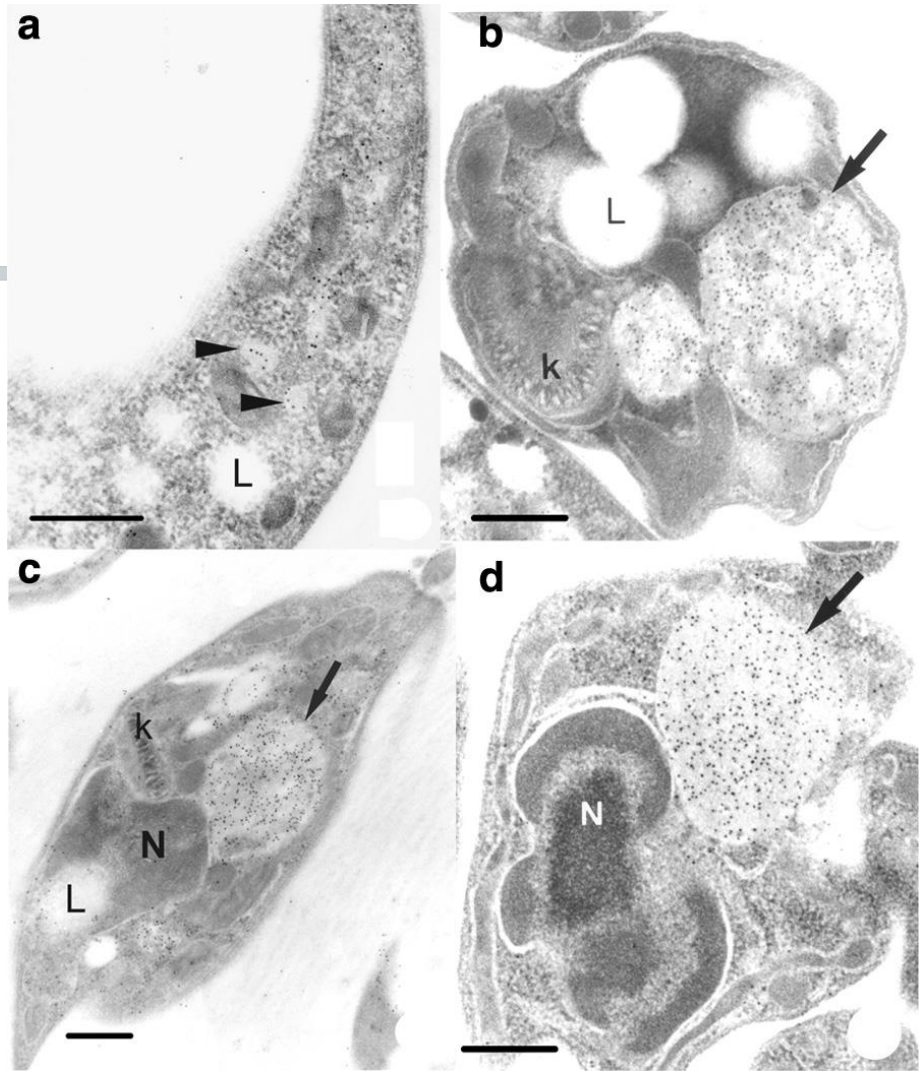
Mcmahon-Pratt et al, 2010

Amastigotes



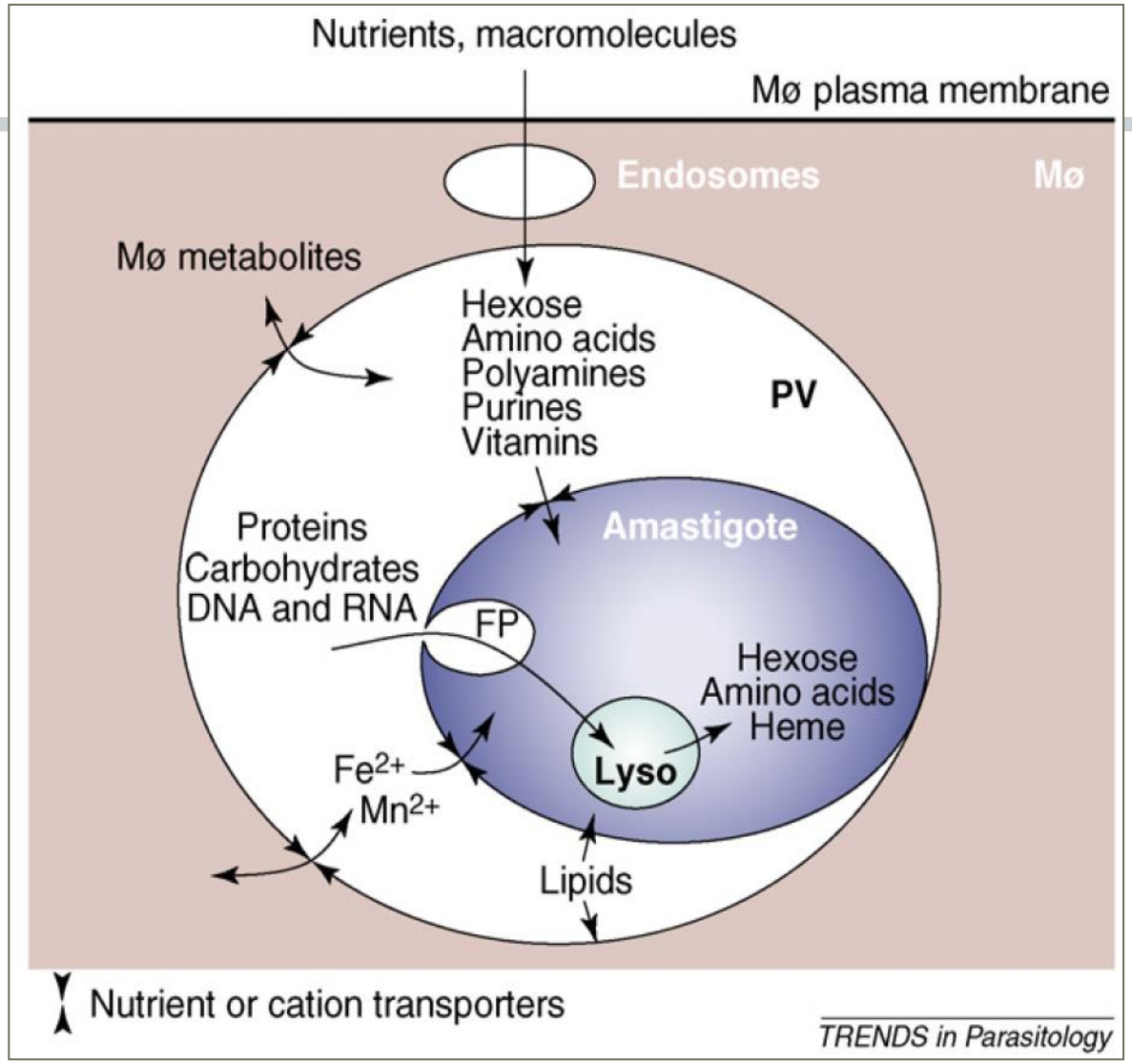
Megasomes in *Leishmania.* Diane McMahon-Pratt, Tania Ueda-Nakamura, and Yara M. Traub-Cseko; in W. de Souza (ed.), *Structures and Organelles in Pathogenic Protists*, Microbiology Monographs 17, Springer-Verlag Berlin Heidelberg, 2010

Ueda-Nakamura et al. 2001



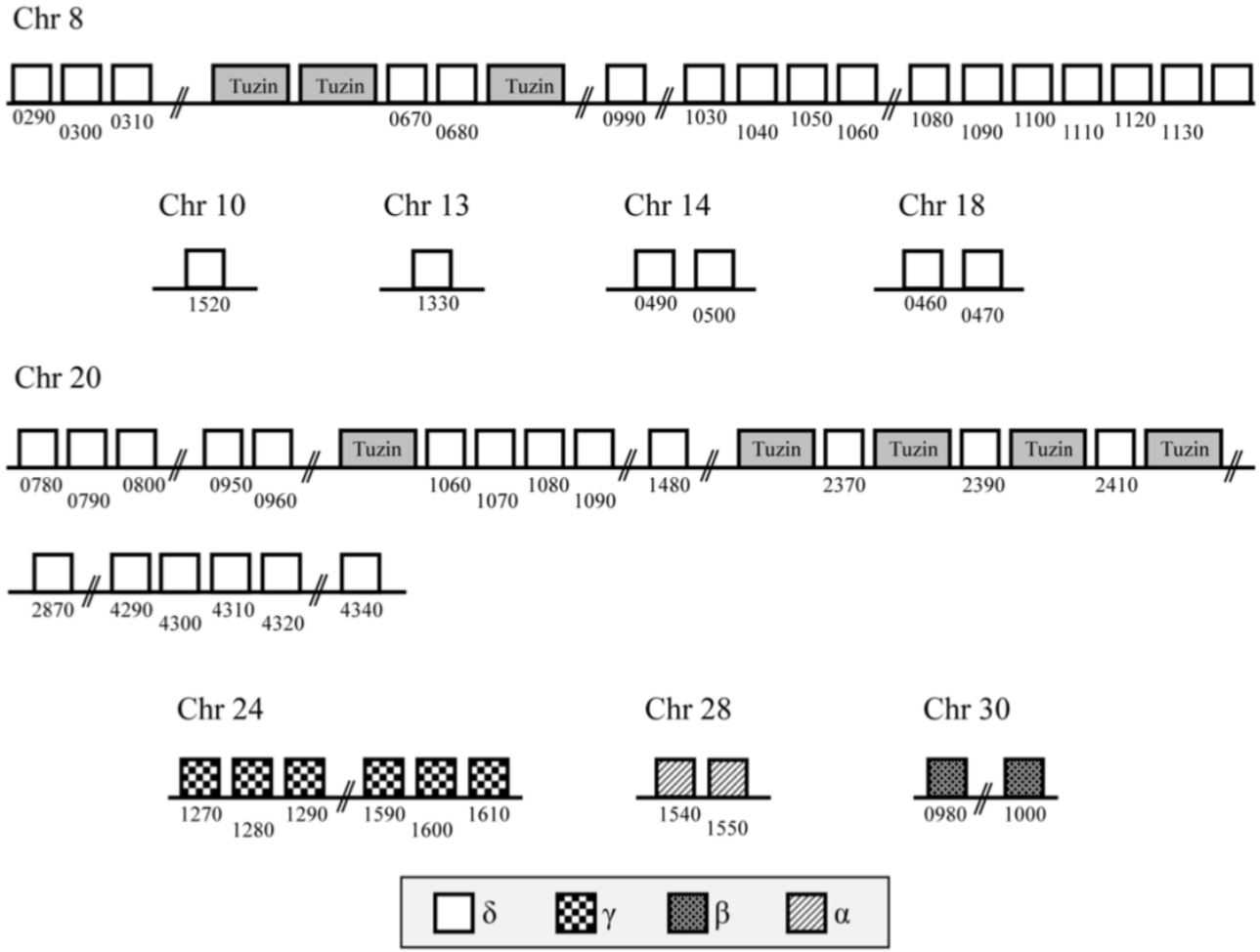
Immunolocalization of cysteine proteinase Lpcys2 by transmission electron microscopy: (a) lysosomes (arrowheads) in promastigote forms of *L. mexicana*; megasomes (arrows) are shown in axenic amastigotes of *L. mexicana* (b), *L. pifanoi* (c), and *L. amazonensis* (d); L lipid inclusion; N nucleus; k kinetoplast; Bars . 1 micra.

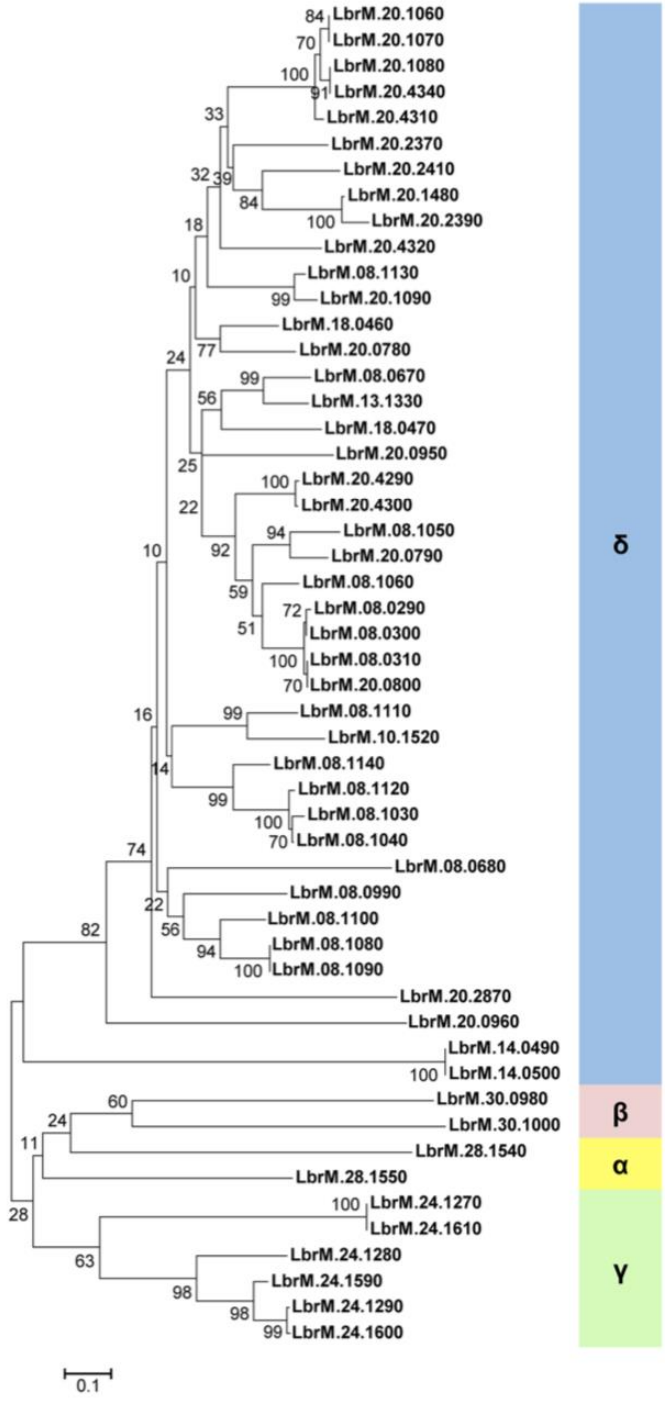
… and metabolically speaking?



Naderer & McConville, 2009

Amastin Knockdown in *Leishmania braziliensis* Affects Parasite-Macrophage Interaction and Results in Impaired Viability of Intracellular Amastigotes. de Paiva RMC, Grazielle-Silva V, Cardoso MS, Nakagaki BN, Mendonça-Neto RP, Canavaci AMC, et al. (2015) **PLoS Pathog 11(12): e1005296.** 10.1371/journal.ppat.1005296





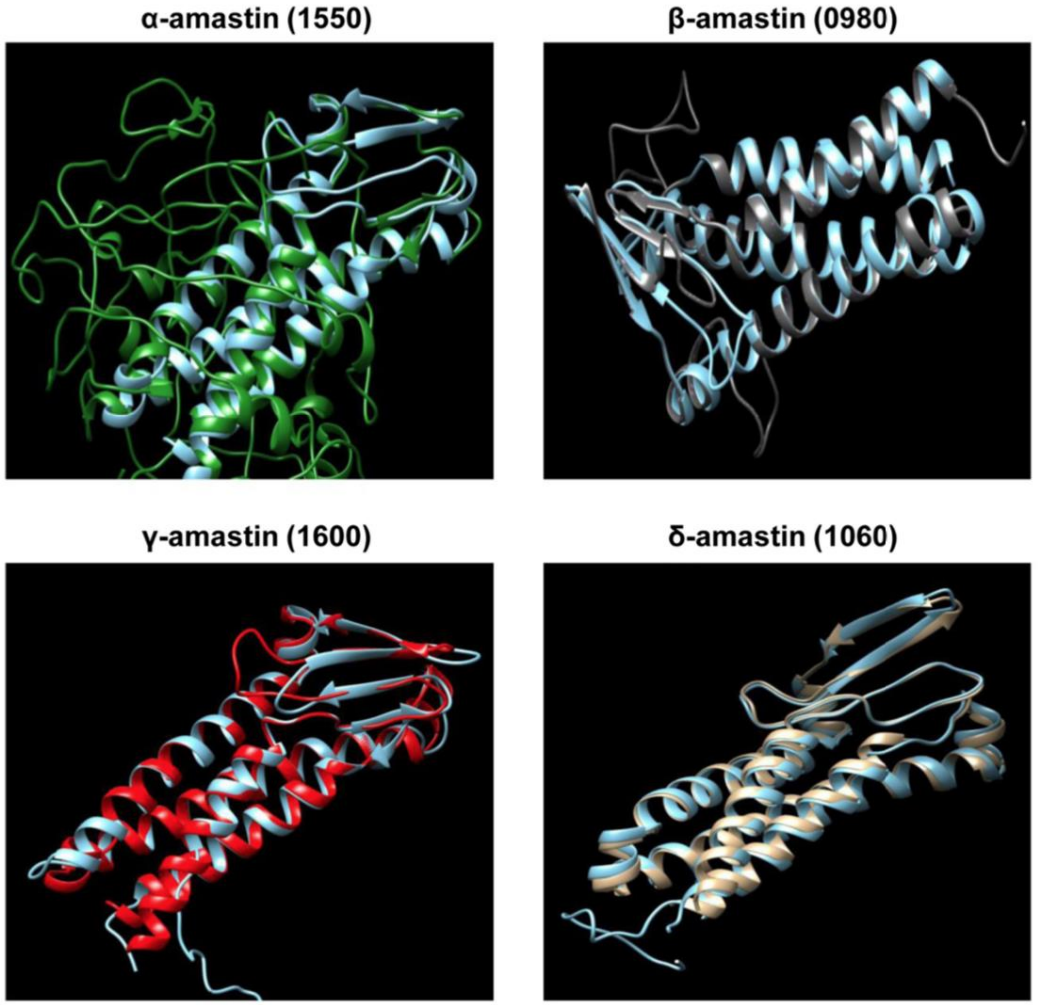


Fig 3. Homology-based 3D modeling of α, β, γ and δ amastins. Structural predictions were done using PHYRE web server and the predicted structures of α, β, γ and δ amastins, were imaged using the UCFS Chimera program. α-amastin is shown in green, β-amastin is shown in gray, γ-amastin is shown in red, δ-amastin is shown in yellow and the superimposed mouse claudin 15 model is shown in blue.

doi:10.1371/journal.ppat.1005296.g003

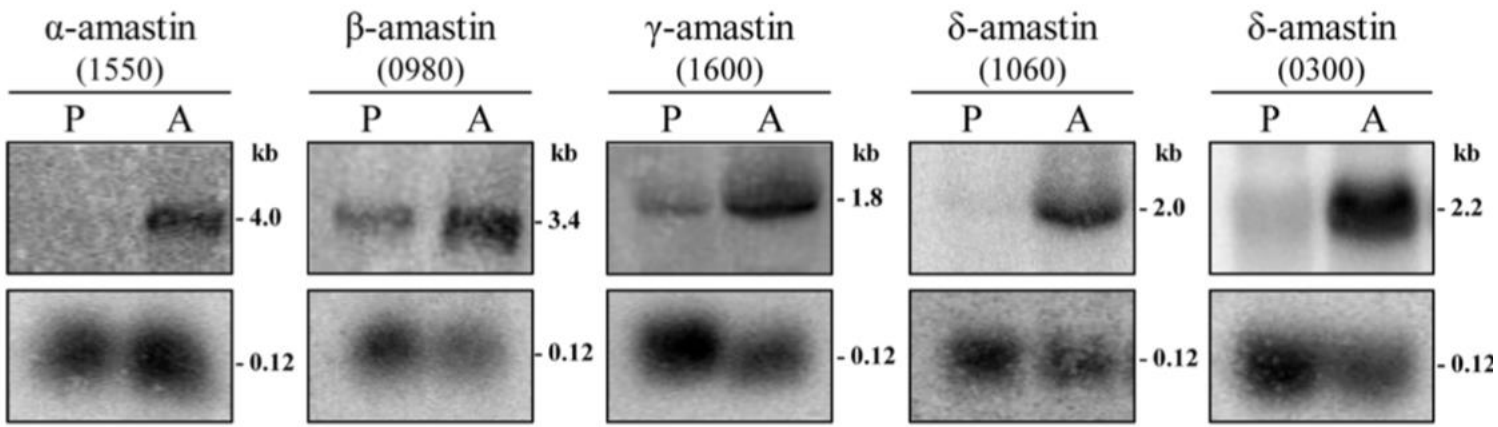


Fig 4. Differential expression of amastin mRNA during the *L. braziliensis* life cycle. Total RNA (10 μg/lane), extrated from promatigote (P) and axenic amastigote (A) forms were separated by electrophoresis, transfered to nylon membranes and probed with the ^{32}P-labelled sequences corresponding to an α-amastin (LbM.28.1550), β-amastin (LbM.30.0980), γ-amastin (LbrM.24.1600) and two δ-amastins (LbM.08.0300 and LbrM.20.1060). Bottom panels show hybridization of the same membranes with a fragment of the 5S rRNA.

doi:10.1371/journal.ppat.1005296.g004

What else?

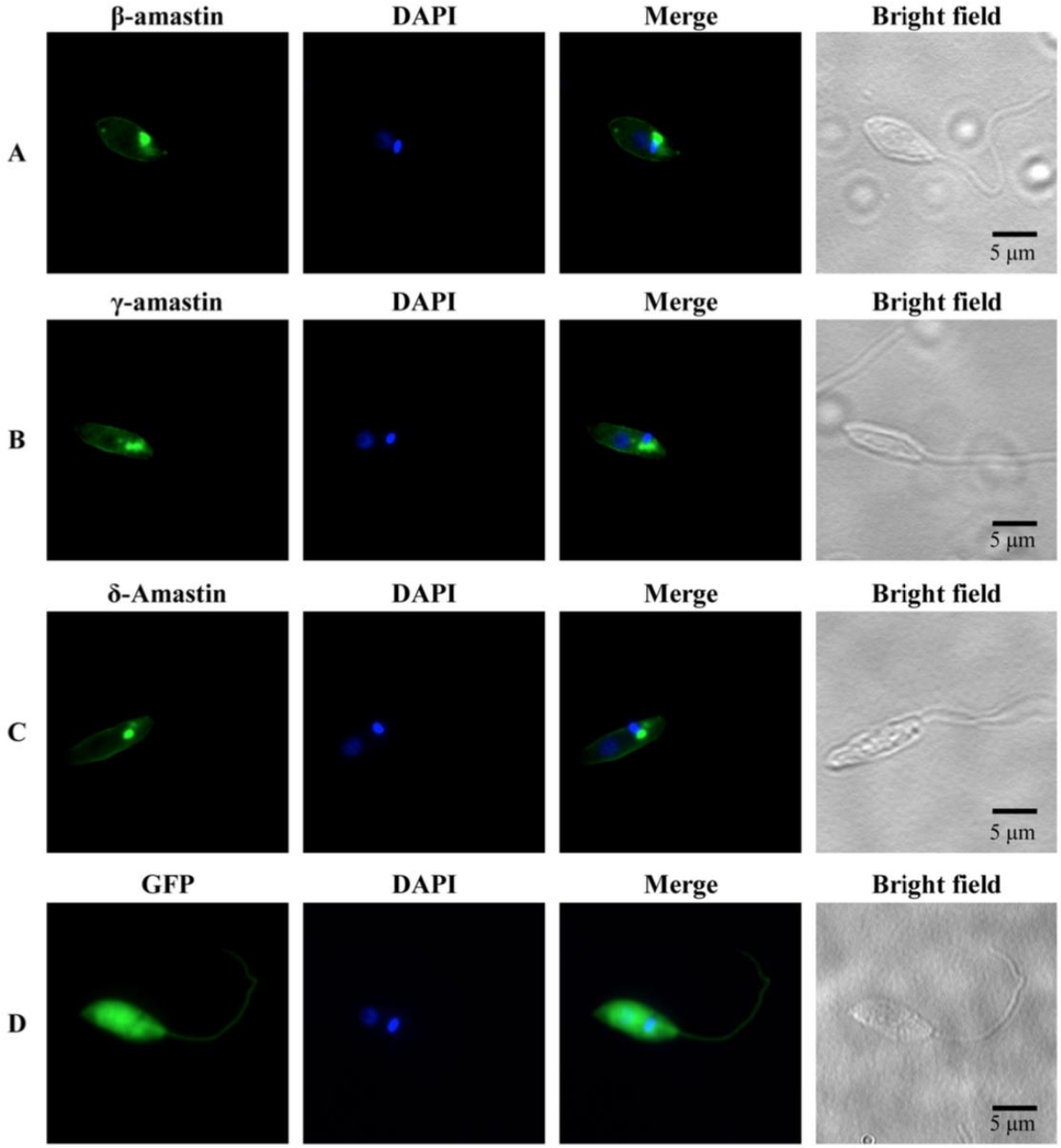


Fig 5. Subcellular localization of distinct amastins in fusion with GFP. Promastigotes were transiently transfected with the plasmids pSP-Ama0980-GFP (A), pSP-Ama1600-GFP (B), pSP-Ama1060-GFP (C) and pSP-nGFP (D) as a control plasmid. Transfected parasites were fixed with 2% paraformaldehyde, stained with DAPI and visualized under a fluorescence microscope. Nuclear and kinetoplast DNA are shown in blue.

doi:10.1371/journal.ppat.1005296.g005

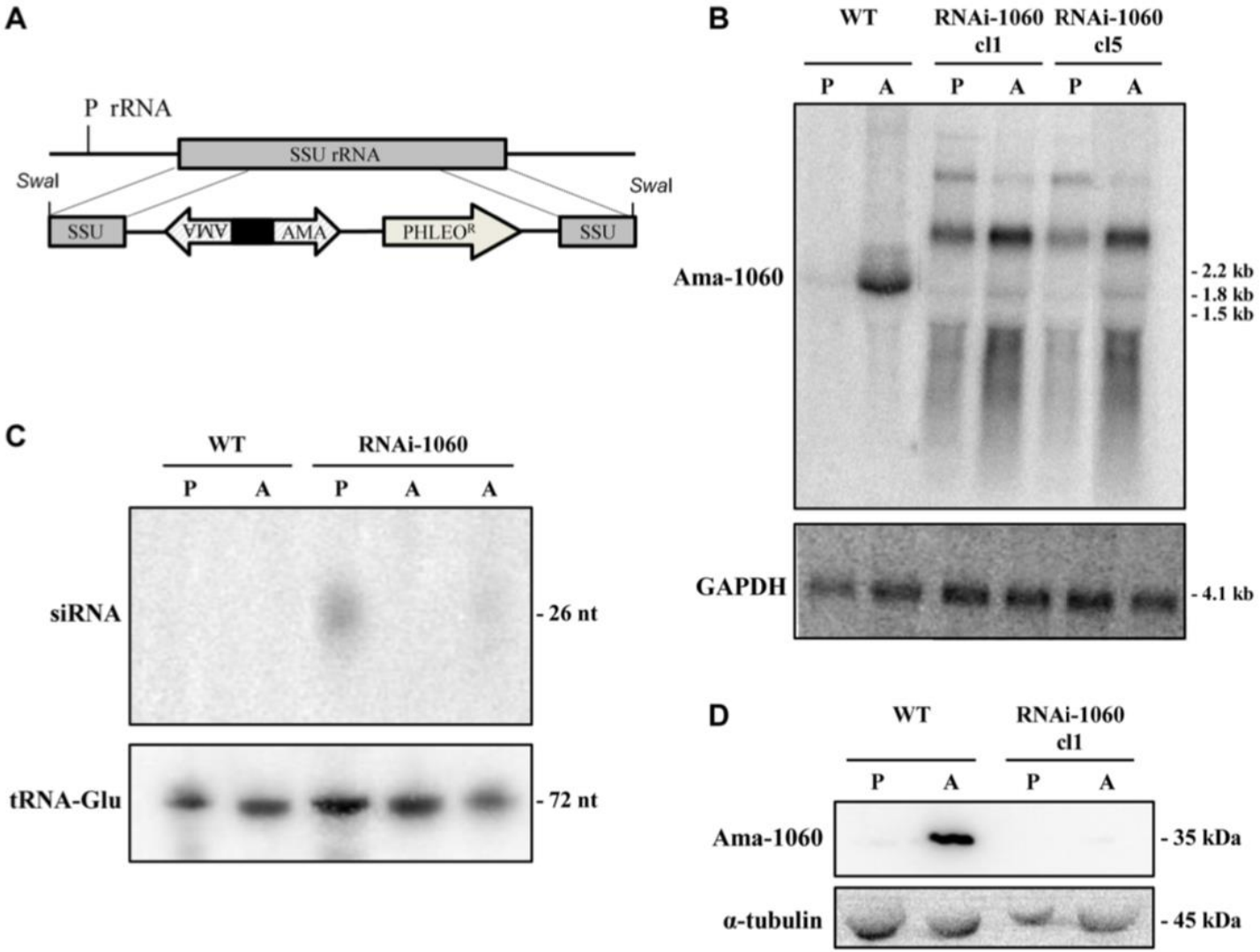


Fig 6. RNAi knockdown of amastin genes in *Leishmania braziliensis*. (A) The pIR1-Phleo plasmid containing two opposite amastin fragments with a stem-loop stuffer fragment (black box), Phleomycin resistance (gene PHLEO) and the rRNA promoter (P rRNA) is shown integrated into the SSU rRNA locus of *Leishmania* (gray box). (B) Northern blot analyses of RNA isolated from (P) promastigote and (A) axenic amastigotes from wild type *L. braziliensis* (WT) and two cloned cell lines of *L. braziliensis* transfected with a construct that generates δ-amastin dsRNA named RNAi-1060-cl1 and cl5. The blots were probed with a ^{32}P-labelled DNA fragment corresponding to the LbrM.08.1060 amastin gene. (C) Low-molecular-weight RNAs isolated from promastigotes and amastigotes from WT *L. braziliensis* as well as from promastigotes from RNAi-1060 cl1 and amastigotes from the two cloned cell line transfected with amastin dsRNA constructs (RNAi-1060 cl1 and cl5) were fractionated on a 15% polyacrylamide gel and probed with a mixture of ^{32}P-labelled oligonucleotide probes corresponding to the full length LbrM.08.1060 amastin gene. siRNA indicates the position of small interfering RNA bands that hybridized with δ-amastin oligonucleotide probes, which co-migrate with a 26 nt DNA molecular weight marker. Hybridization of the same blot with a probe corresponding to the *L. braziliensis* Glu-tRNA is also shown as a loading control. (D) Total protein extracts from the cloned cell RNAi-1060 cl1 was analyzed by western blot using an antibody generated against the recombinant Ama1060. The same blot was incubated with anti-α-tubulin as a loading control.

doi:10.1371/journal.ppat.1005296.g006

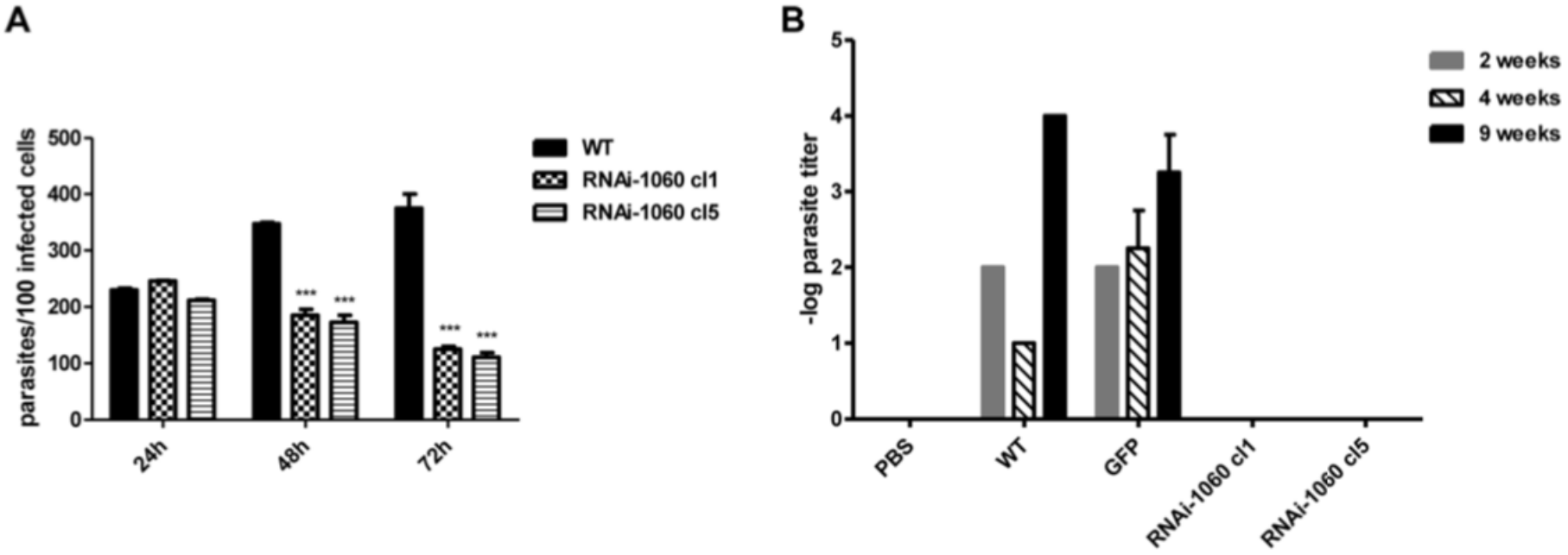


Fig 7. Infection of mouse macrophages and BALB/c mice footpads with WT *L. braziliensis* and RNAi-1060 cell lines. (A) Intraperitoneal macrophages from BALB/c mice were incubated for 24 hours at 34°C with stationary phase promastigotes from WT *L. braziliensis* cultures and the two cloned cell lines RNAi-Ama1060 cl1 and cl5 at a ratio of 10: 1 parasites/cell. After washing non-internalized promastigotes macrophages were incubated for 24, 48 or 72 hours before the cells were stained with DAPI and the numbers of intracellular amastigotes, visualized by fluorescence microscopy, were determined. (B) BALB/c mice were infected in the footpads with 10^7 stationary phase promastigotes from WT *L. braziliensis*, *L. braziliensis* transfected with the pIR1PHLEO vector containing GFP, and two cloned cell lines expressing δ-amastin siRNA, RNAi-1060cl1 and cl5. Two, four and nine weeks after infection, parasitism was evaluated by the limiting dilution method.

doi:10.1371/journal.ppat.1005296.g007

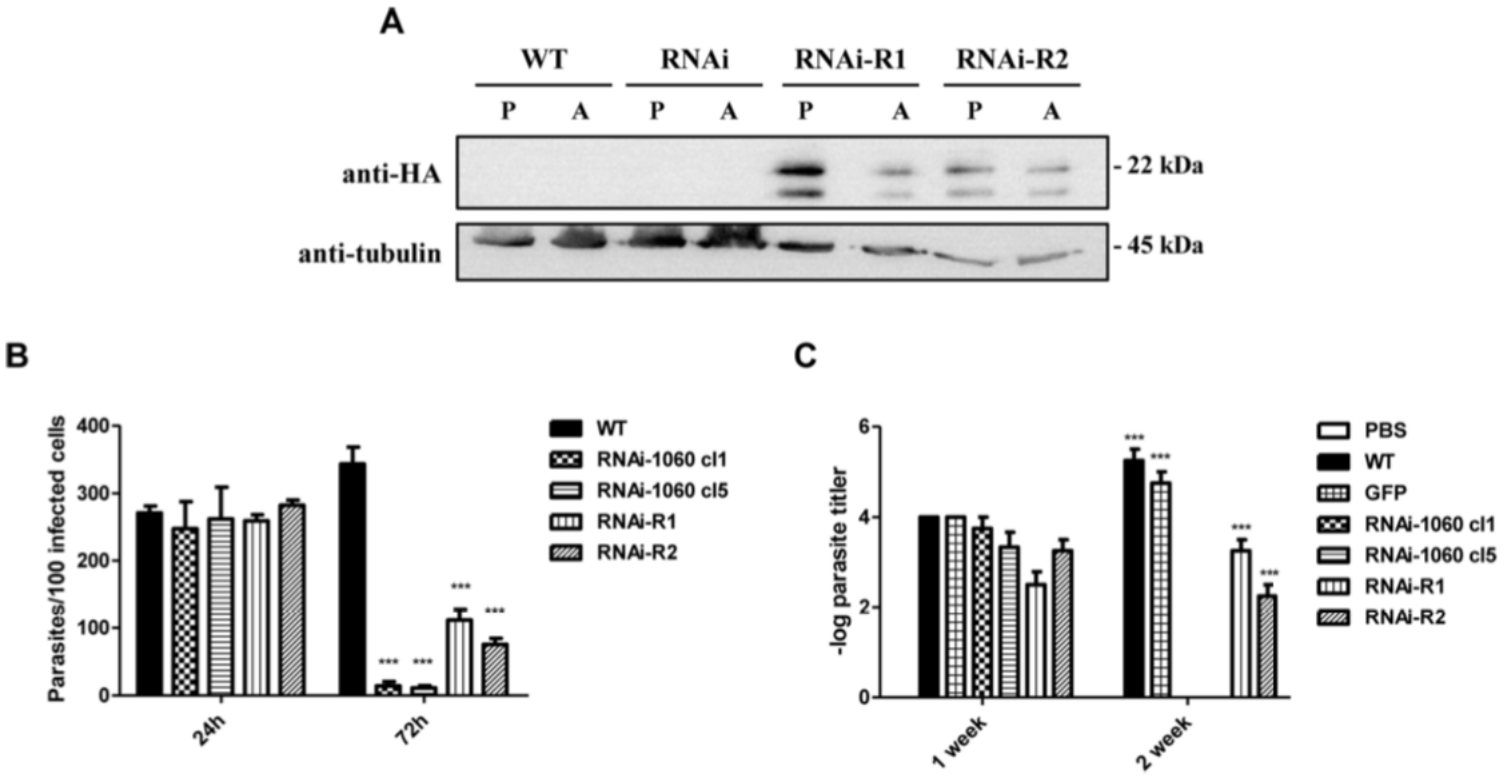
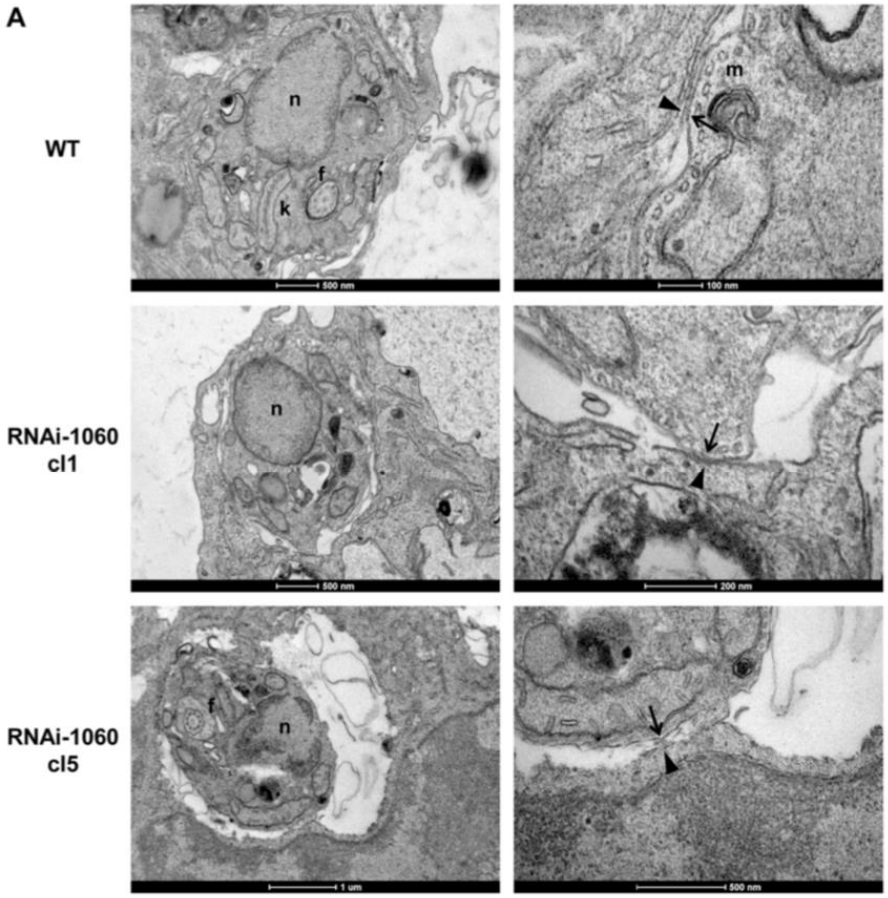


Fig 8. Re-expression of amastin sequences in RNAi knockdown parasites rescue infection capacity of *L. braziliensis*. (A) Total protein extracts from promastigotes (P) and amastigotes (A) of WT *L. braziliensis*, the cloned cell RNAi-1060 cl1 and two cloned cell lines derived from the RNAi-1060 parasites the were transfected with an RNAi-resistant amastin gene (RNAi-1060-R2 and RNAi-1060-R4) were analysed by western blot using an anti-HA antibody. Bands corresponding to the Ama1060 synthetic gene containing the HA epitope are shown only in the re-expressor cell lines. The same blot was incubated with anti-tubulin antibody as a loading control. (B) Stationary phase promastigotes from WT *L. braziliensis*, two cloned cell lines expressing amastin dsRNA, RNAi-1060 cl1 and cl5 and two cloned cell lines that express a RNAi resistant amastin sequence were used to infect BALB/c mice peritoneal macrophages at a ratio of 10:1 (parasites/cell) and the numbers of intracellular amastigotes determined 24 and 72 hours post-infection. (C) Stationary phase promastigotes (10^7 parasites) from WT *L. braziliensis*, *L. braziliensis* transfected with the pIR1PHLEO vector containing GFP, the two cloned cell lines expressing amastin dsRNA, RNAi-1060 cl1 and cl5 and two cloned cell lines that express a RNAi resistant amastin sequence were used to infected BALB/c mice footpads. One or two weeks post-infection, parasitism was evaluated by the limiting dilution of parasites recovered from the mice footpads.

doi:10.1371/journal.ppat.1005296.g008

Infection time course

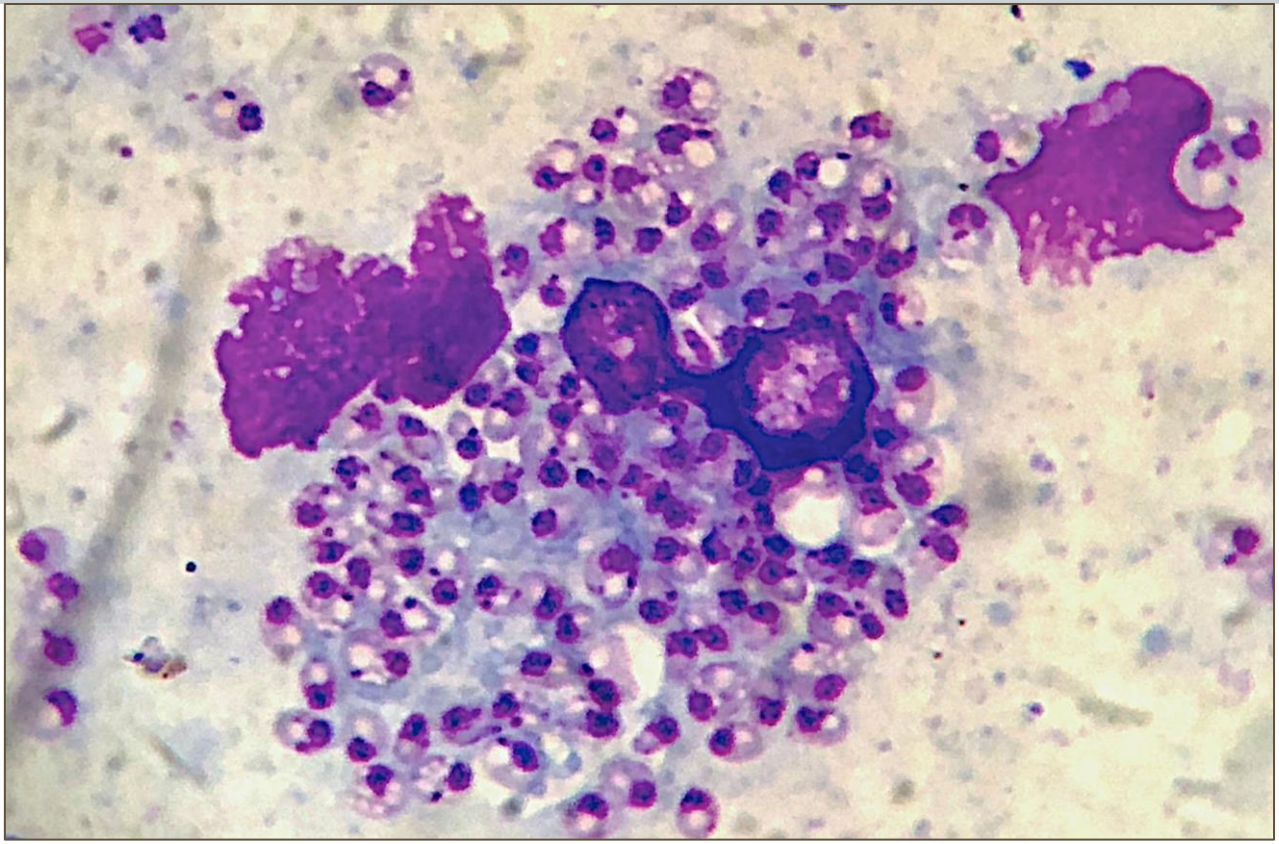

A
WT
RNAi-1060 cl1
RNAi-1060 cl5
n
f
k
m
500 nm
100 nm
200 nm
1 um
B
Ratio (vacuole area/parasite area)
2.5
2.0
1.5
1.0
0.5
0.0
***
*** p<0.0001
WT
RNAi-1060 cl1
RNAi-1060 cl5

So…

The Role of IL-10 in Promoting Disease Progression in Leishmaniasis

Margaret Mentink Kane and David M. Mosser

J Immunol, 2001, 166 (2) 1141-1147
DOI:10.4049/jimmunol.166.2.1141



"In the present study, we examined cytokine production by macrophages following their interaction with Leishmania amastigotes".

The Role of IL-10 in Promoting Disease Progression in Leishmaniasis

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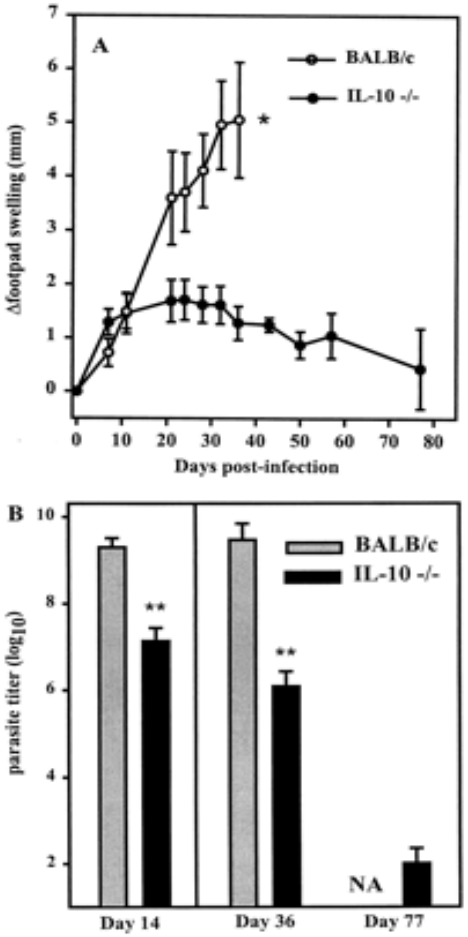
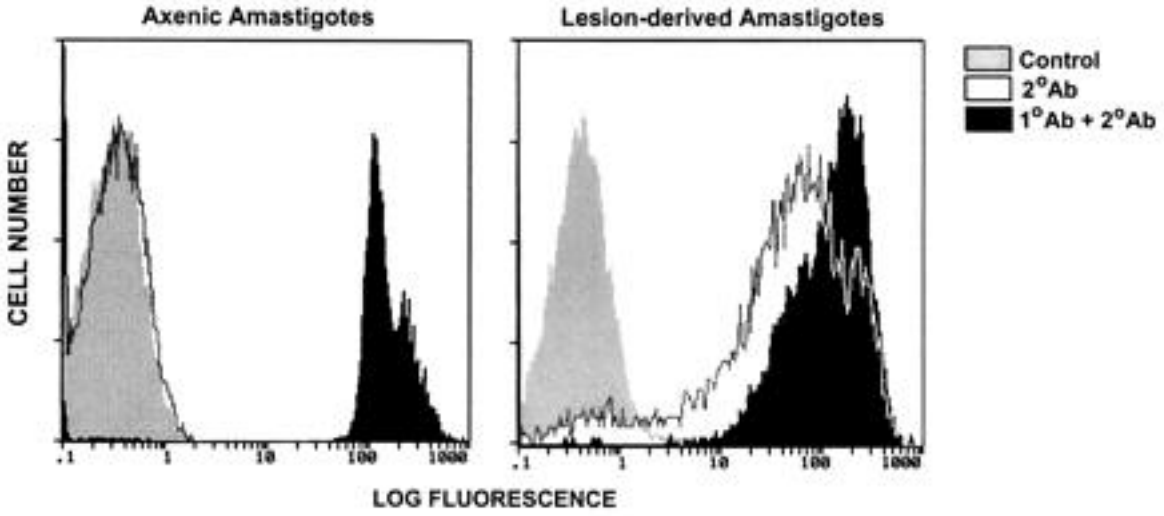
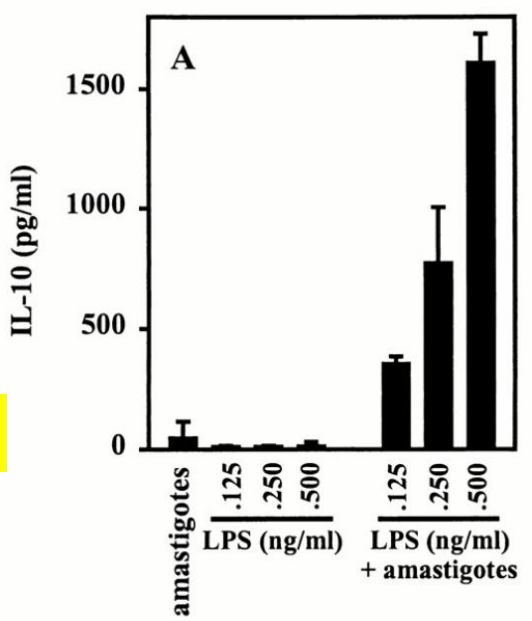
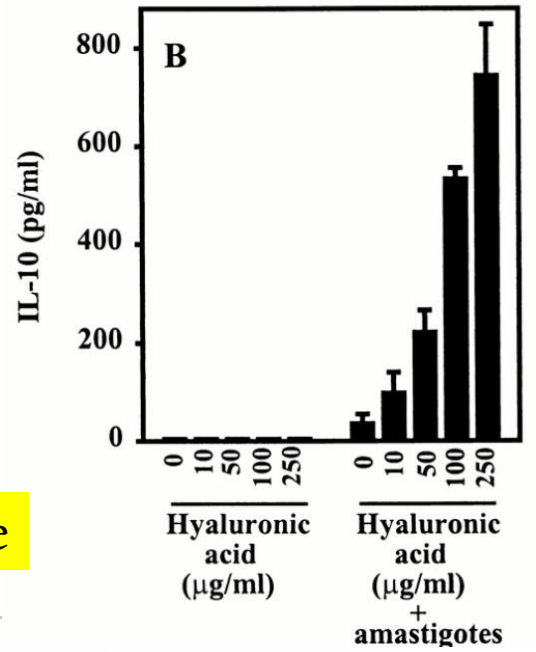




moi

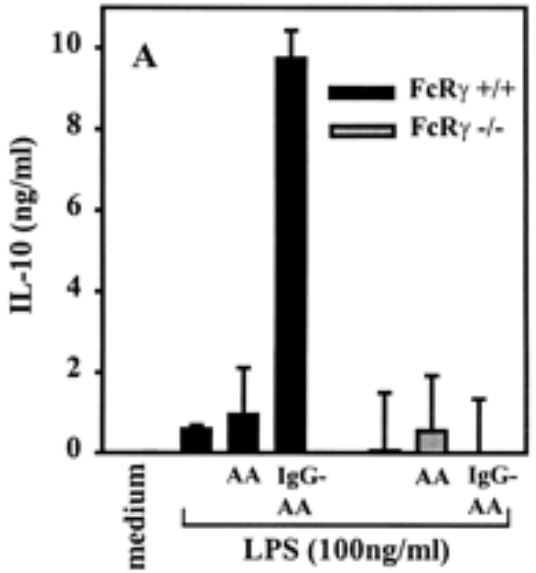


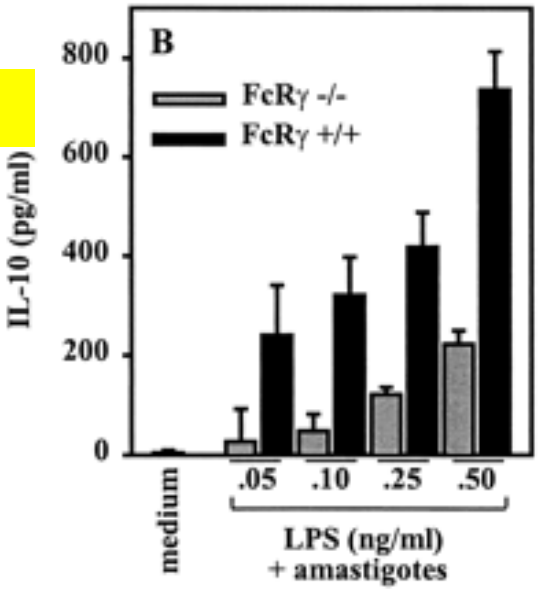


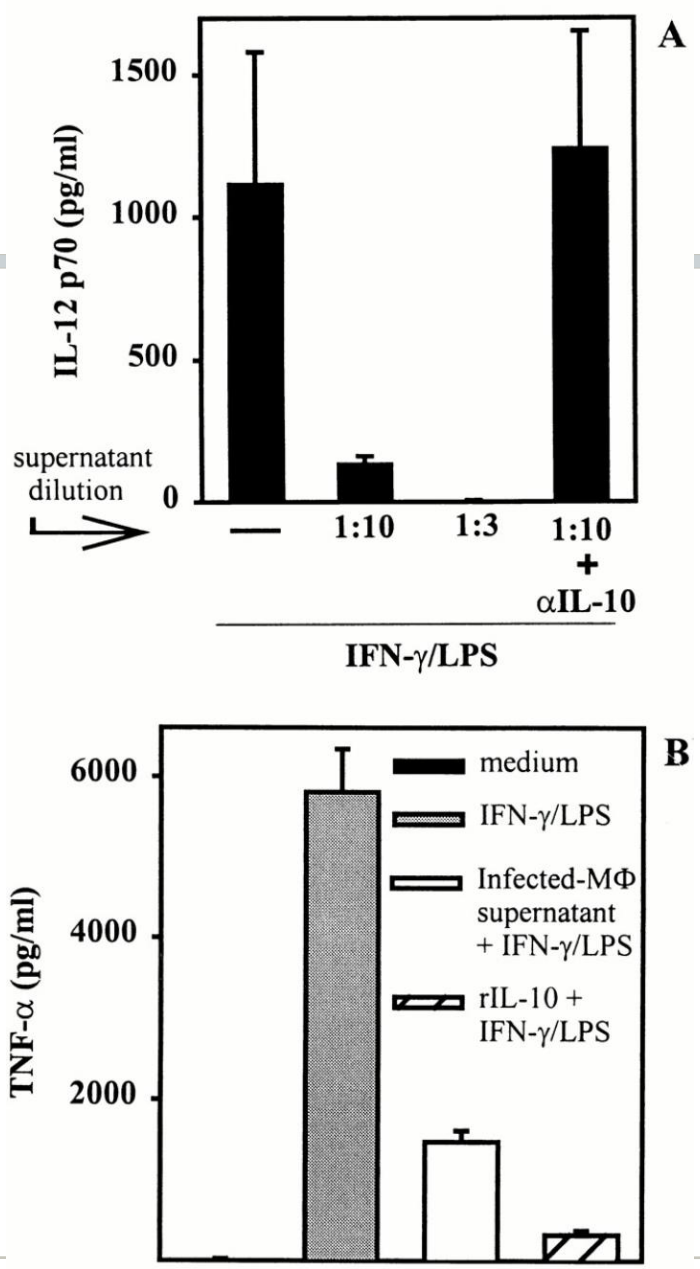
scale

Previous studies → FcgR ligation could induce the secretion of IL-10 from stimulated macrophages

"Thus, maximal IL-10 production by macrophages infected with Leishmania *amastigotes requires FcgR ligation along with a second costimulatory signal, such as bacterial products or components of the extracellular matrix"*

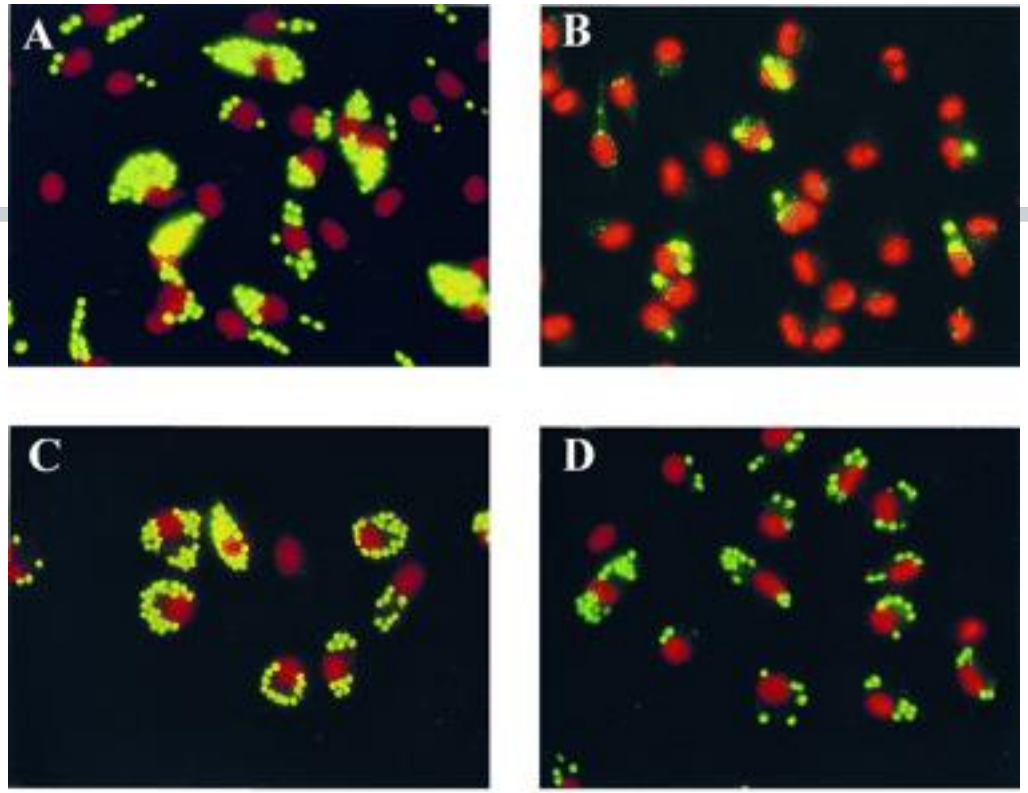


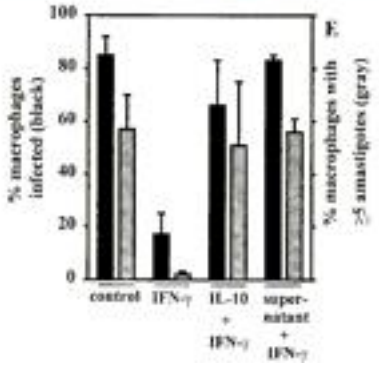




"Supernatants from BMM stimulated with amastigotes in the presence of LPS for 20 h (infected macrophage supernatants) were harvested and diluted 1/3 or 1/10 (v/v) with complete medium"

"These results indicate that IL-10 produced by amastigote-infected inflammatory macrophages is adequate to inhibit the production of both IL-12 and TNF-a by stimulated macrophages"


A
B
C
D


100
80
60
40
20
0
% macrophages infected (black)
% macrophages with ≥5 amastigotes (gray)
control
IFN-γ
IL-10 + IFN-γ
super-natant + IFN-γ

Take home message…

"Rather than simply acting as a classical opsonin to accelerate parasite phagocytosis, an additional role of surface IgG is to induce the production of IL-10 by macrophages. This induction prevents these cells from responding to IFN-g and eliminating intracellular parasites".

Host response is subverted by *Leishmania*!!!