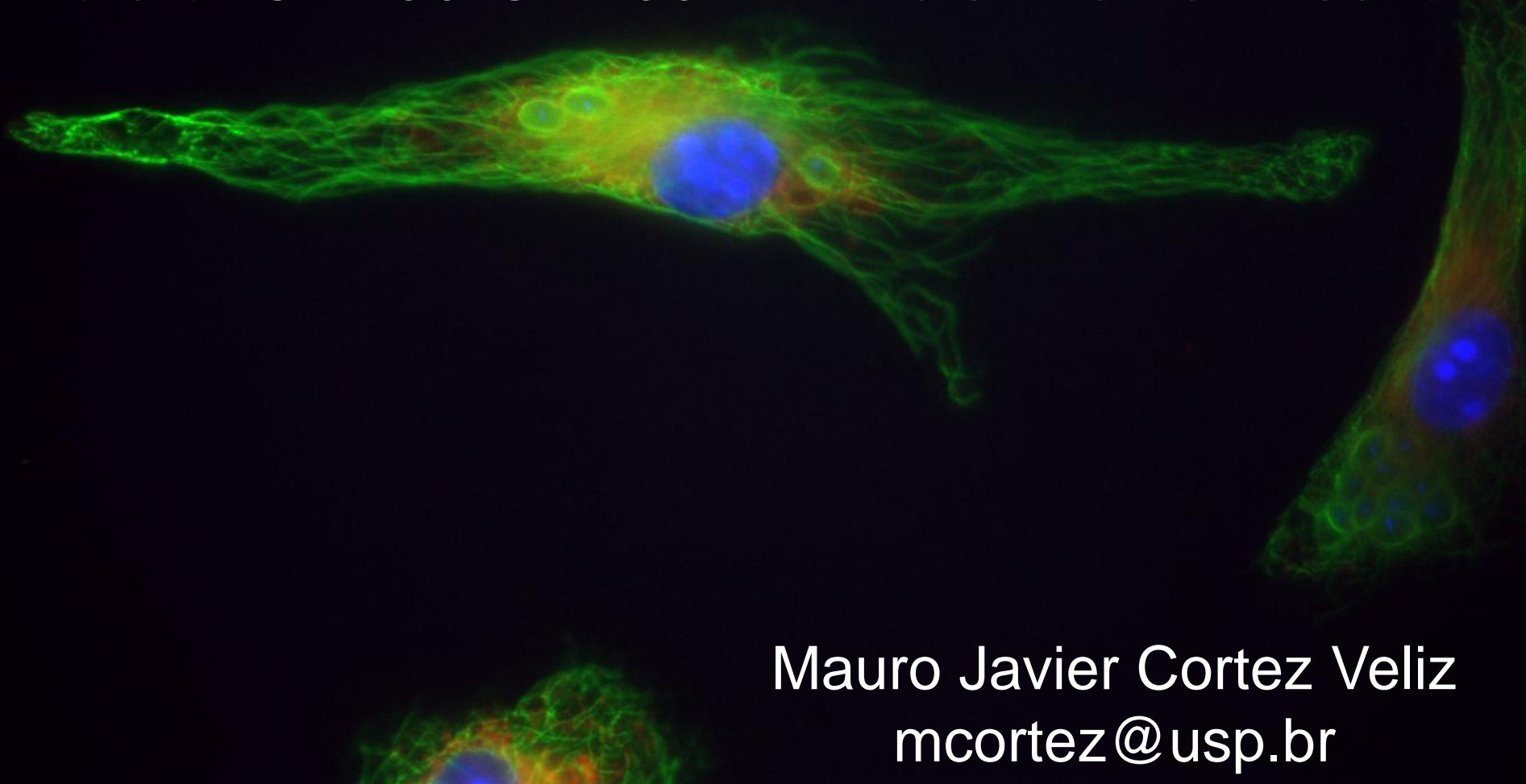


Role of CD200-CD200R interaction in infection of  
macrophage by *Leishmania*

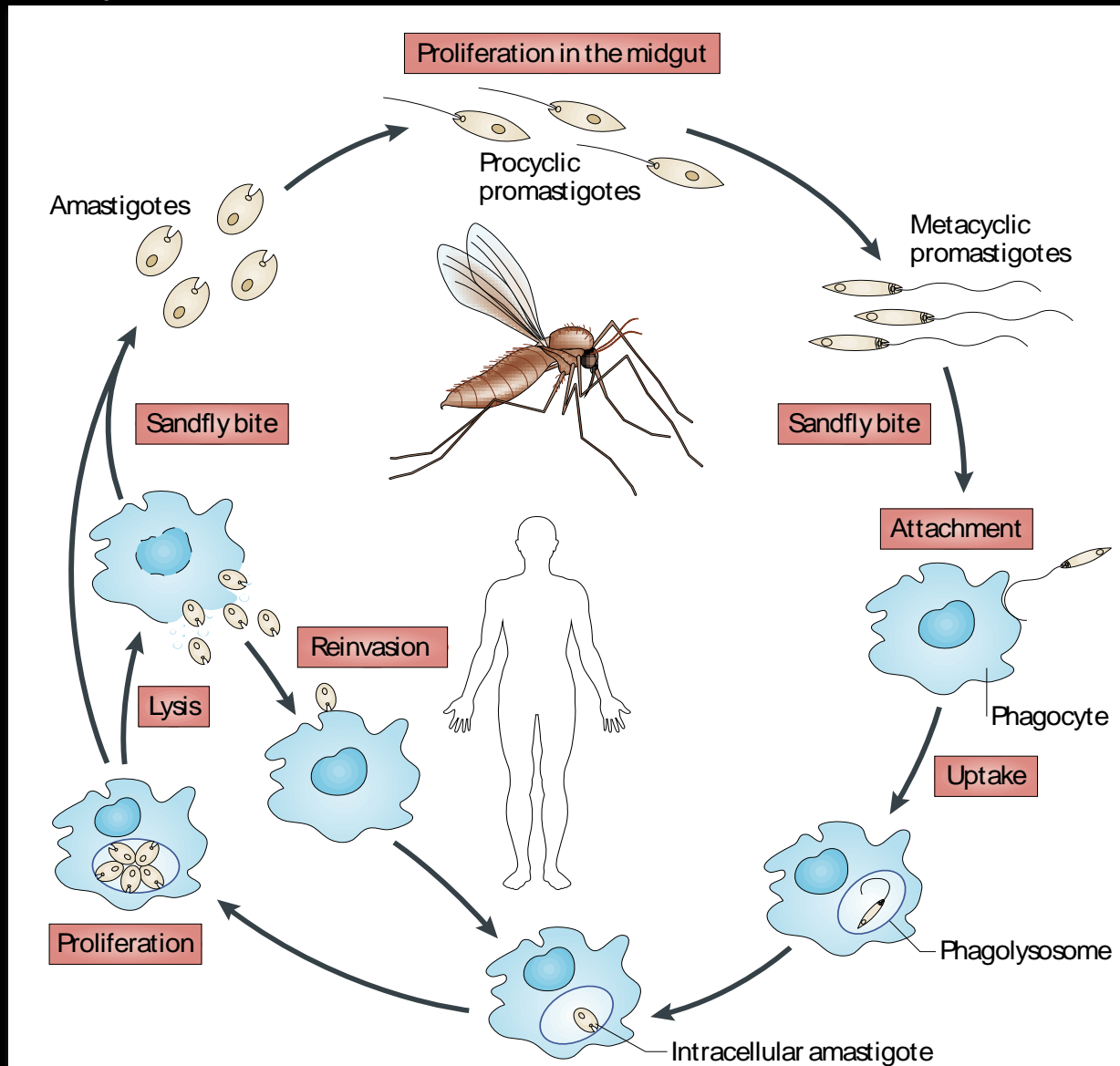
Mauro Javier Cortez Veliz  
mcortez@usp.br

# Role of CD200-CD200R in *Leishmania* infection



Mauro Javier Cortez Veliz  
mcortez@usp.br

# Biological cycle of *Leishmania* ssp



# *Leishmaniasis*



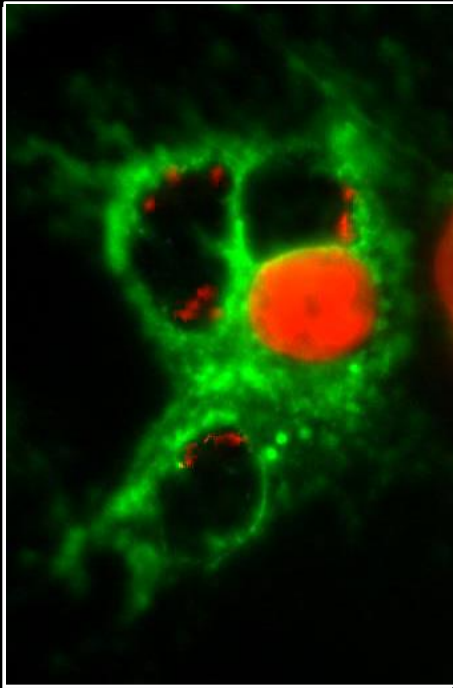
# Leishmania species and leishmaniasis:

Table 1. Leishmania species and associated human diseases

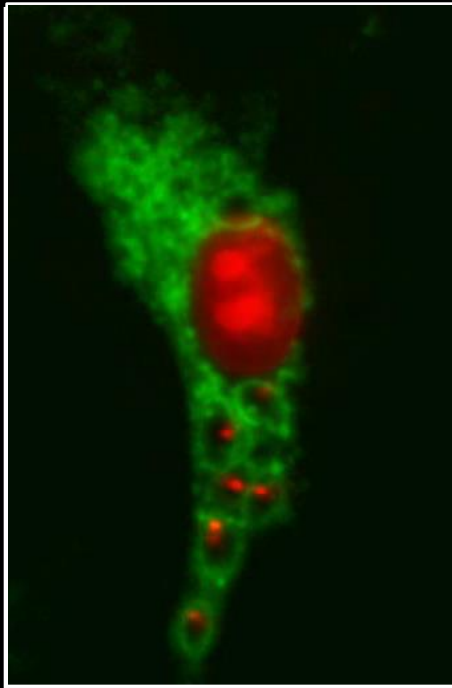
Disease form	New World species		Old World species
Cutaneous	<p>L (L.) mexicana complex</p> <p>L (Viannia) subgenus</p>	<p>{ L (L.) mexicana L (L.) amazonensis L (L.) piñanoi L (L.) venezuelensis L (V.) braziliensis L (V.) peruviana L (V.) lansonii L (V.) naiff L (V.) lansonii L (V.) panamensis L (V.) guyanensis</p>	<p>L (L.) major complex</p> <p>{ L (L.) major L (L.) tropica L (L.) aethiopica</p>
Diffuse cutaneous	<p>L (L.) amazonensis L (L.) piñanoi</p>		<p>L (L.) aethiopica</p>
Mucocutaneous	<p>L (V.) braziliensis</p>		
Visceral	<p>L (L.) donovani complex</p>	<p>{ L (L.) chagasi*</p>	<p>L (L.) infantum* L (L.) donovani</p>

\*Generally, *L. infantum* and *L. chagasi* are the causative agents of visceral leishmaniasis; however, cases of cutaneous leishmaniasis have been reported (214, 215).

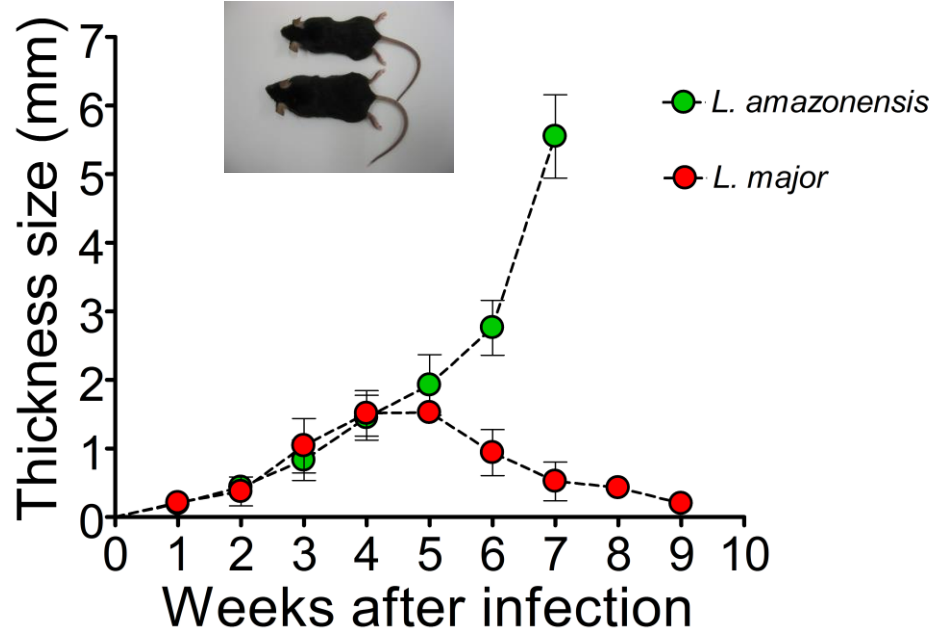
## Cutaneous leishmaniasis species:



*L. (L.) amazonensis*



*L. (L.) major*

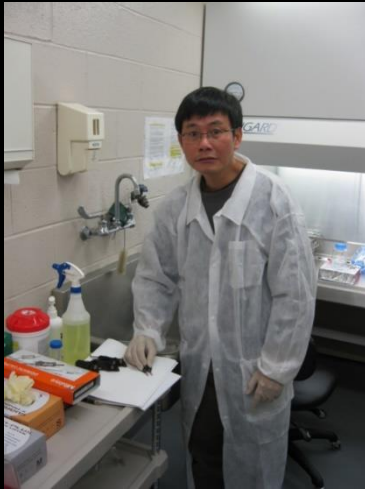


Why *L. (L.) amazonensis* is more virulent than *L. (L.) major*?

# Initial evidence:

## Microarray assay:

-Macrophages (Bone marrow) infected with *Leishmania amazonensis* amastigotes for 48 h:



Chau Huynh

Institute of Systems Biology, Seattle, WA:

Kathy Kennedy

Alan Aderem



## RESULTS:

- Detection of **CD200** up-regulation in infected macrophages.

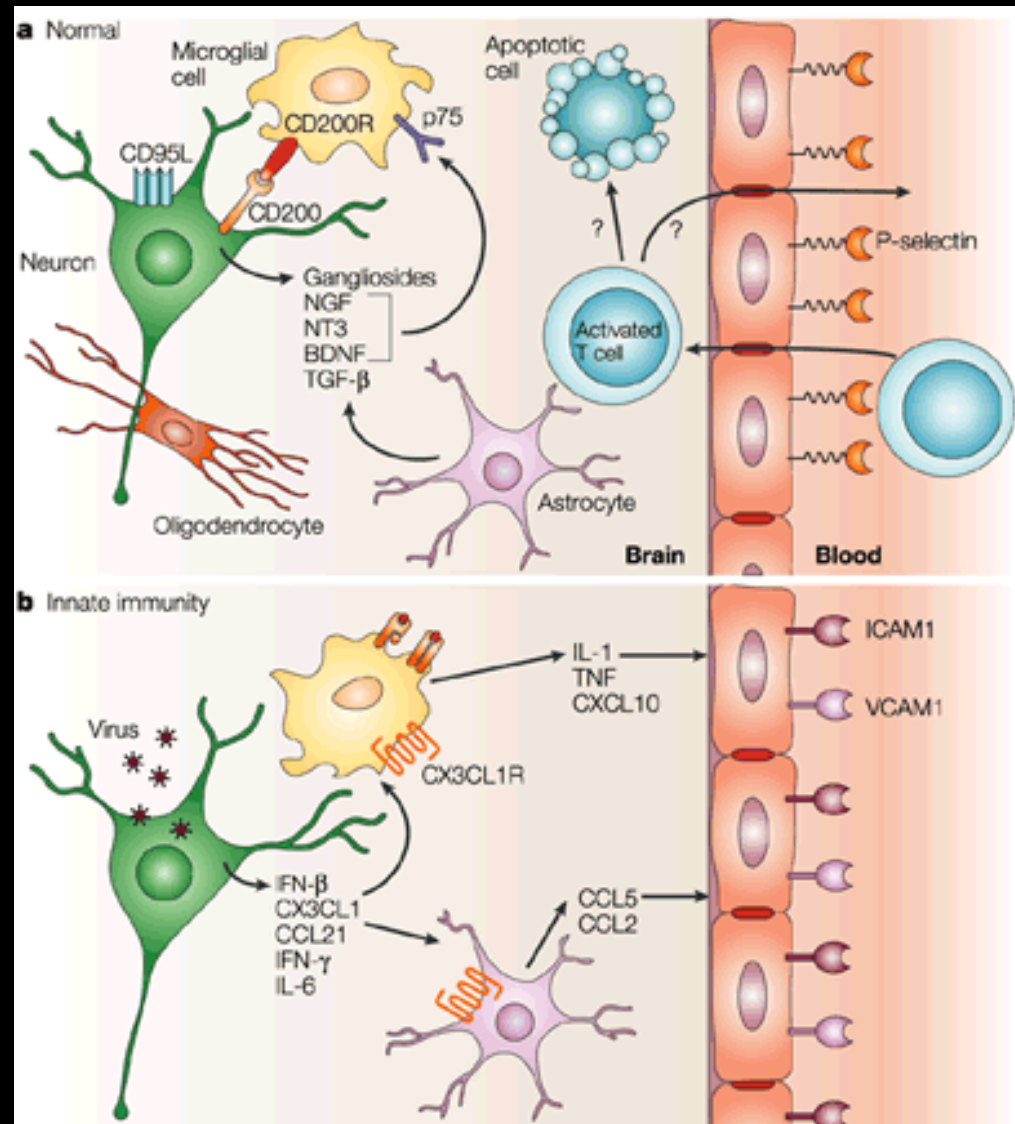
## CD200

- Type 1 Glycoprotein of membrane.
- 2 domains of Ig
- Superfamily of immunoglobulins

Main role:

a. To control macrophage response.

b. To contain exaggerated immune response.



Diane E. Griffin. *Nature Reviews Immunology* (2003)

# Is CD200 induction important for *Leishmania* infection?



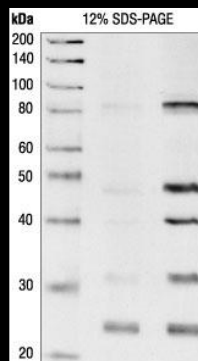
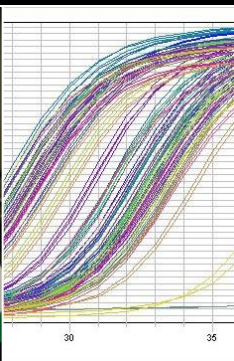
C57BL/6 WT  
Jackson Lab



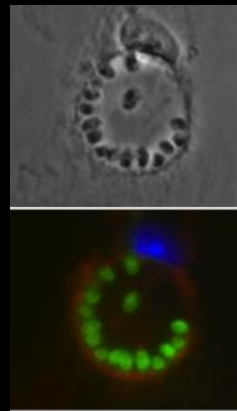
C57BL/6 CD200-/-  
Schering-Plough BioPharma



qPCR



WB

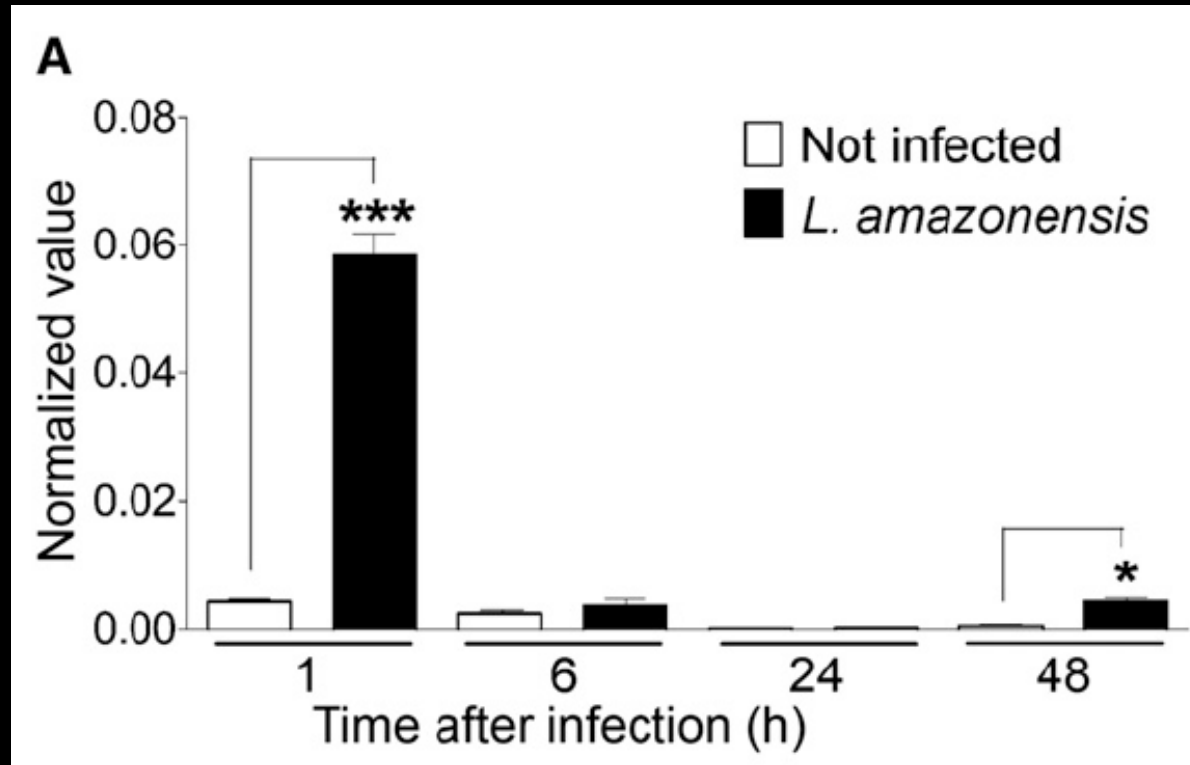


infection *in vitro*

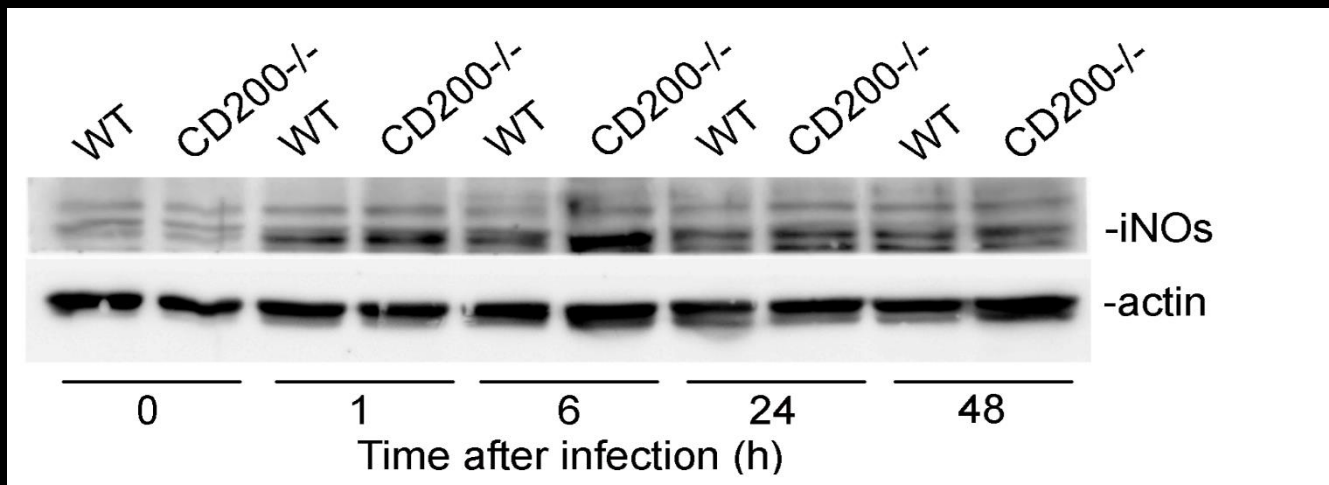
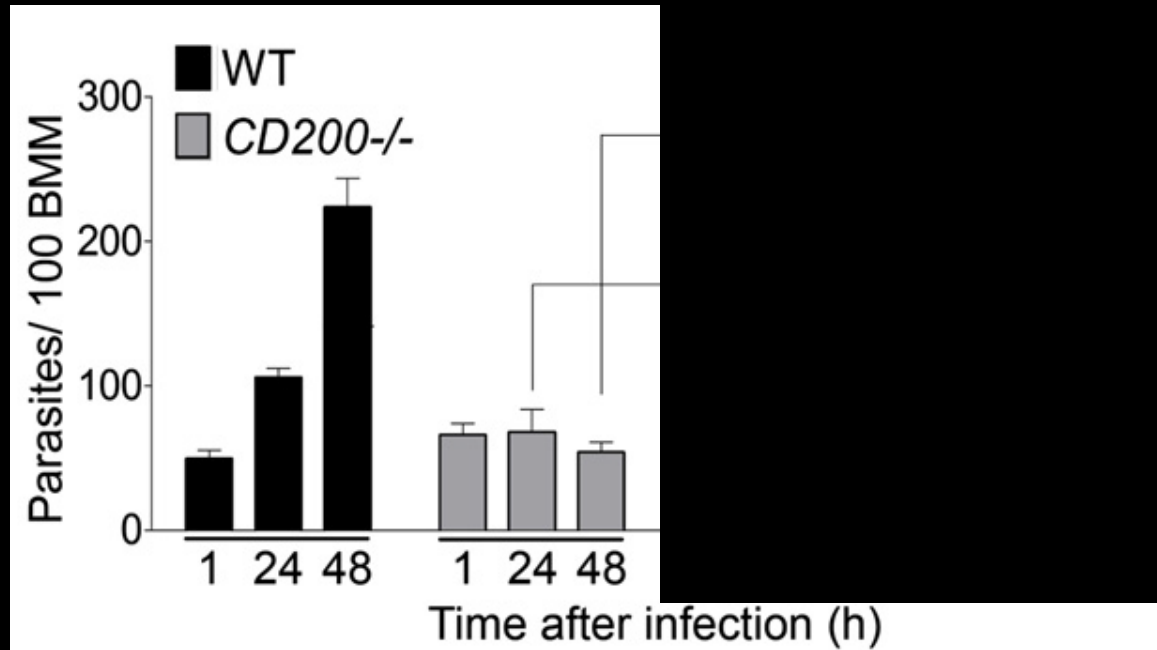


infection *in vivo*

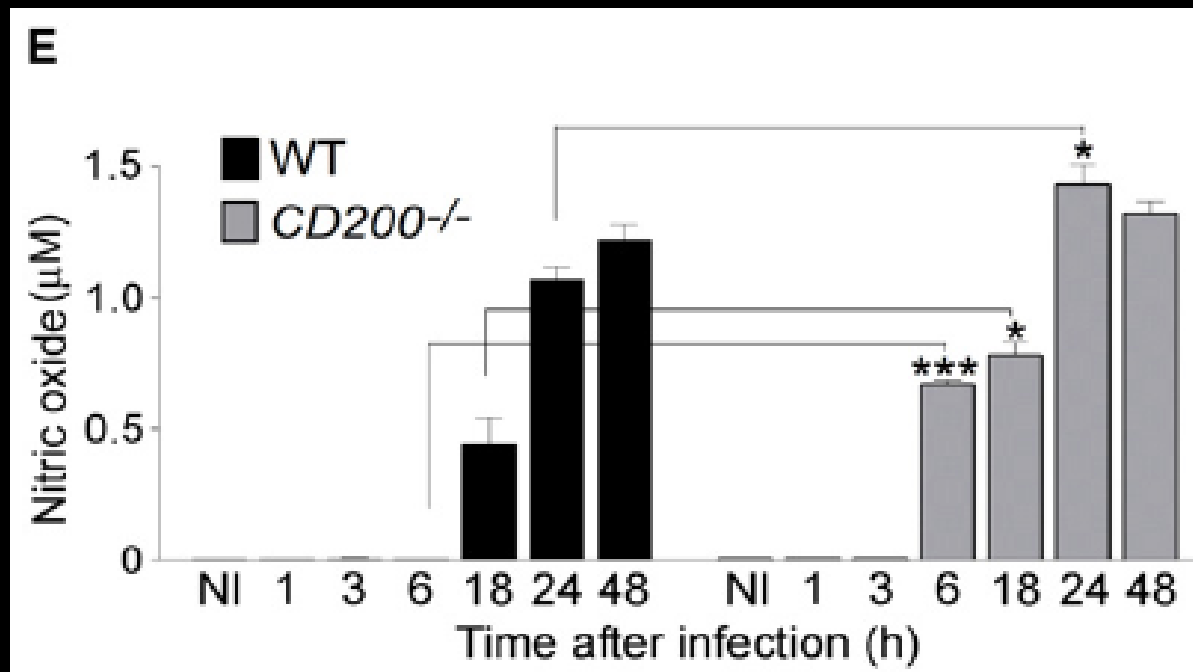
*L. (L.) amazonensis* induces CD200 in bone marrow macrophages (BMM)



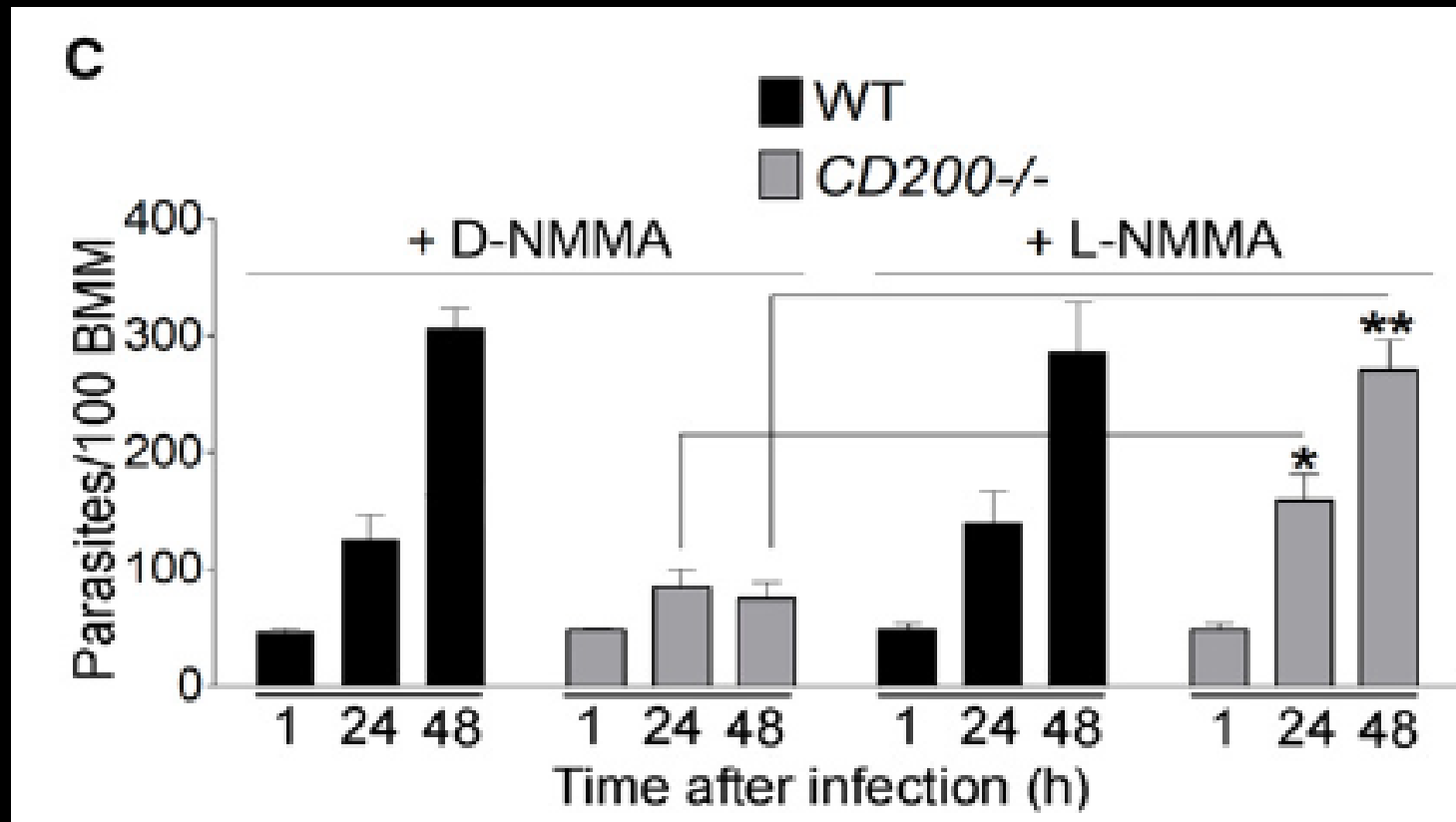
*L. (L.) amazonensis* induces CD200 and inhibits iNOS-NO production - important response to kill parasites



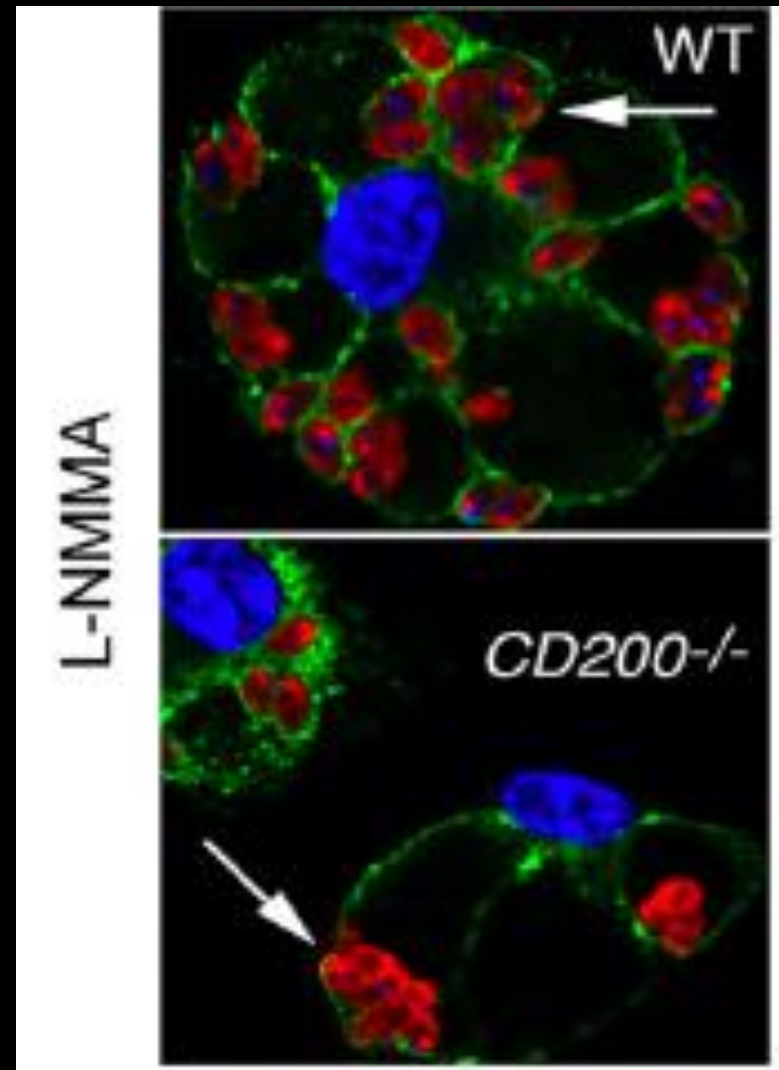
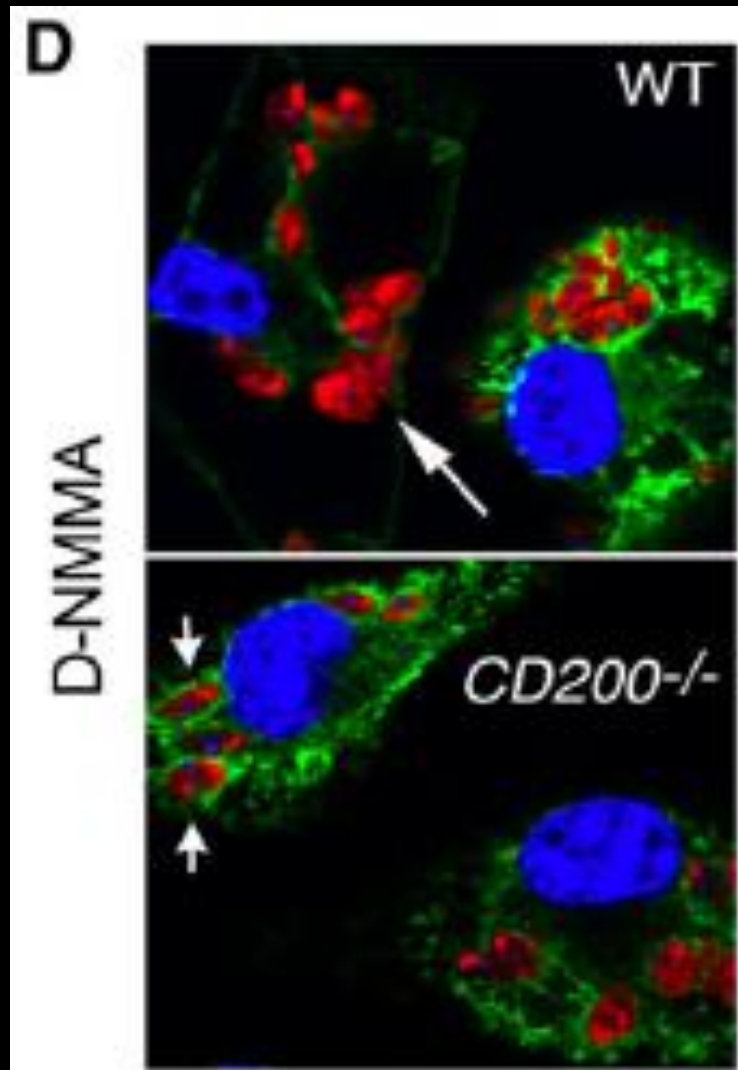
*L. (L.) amazonensis* induces CD200 and inhibits iNOS-NO production - important response to kill parasites



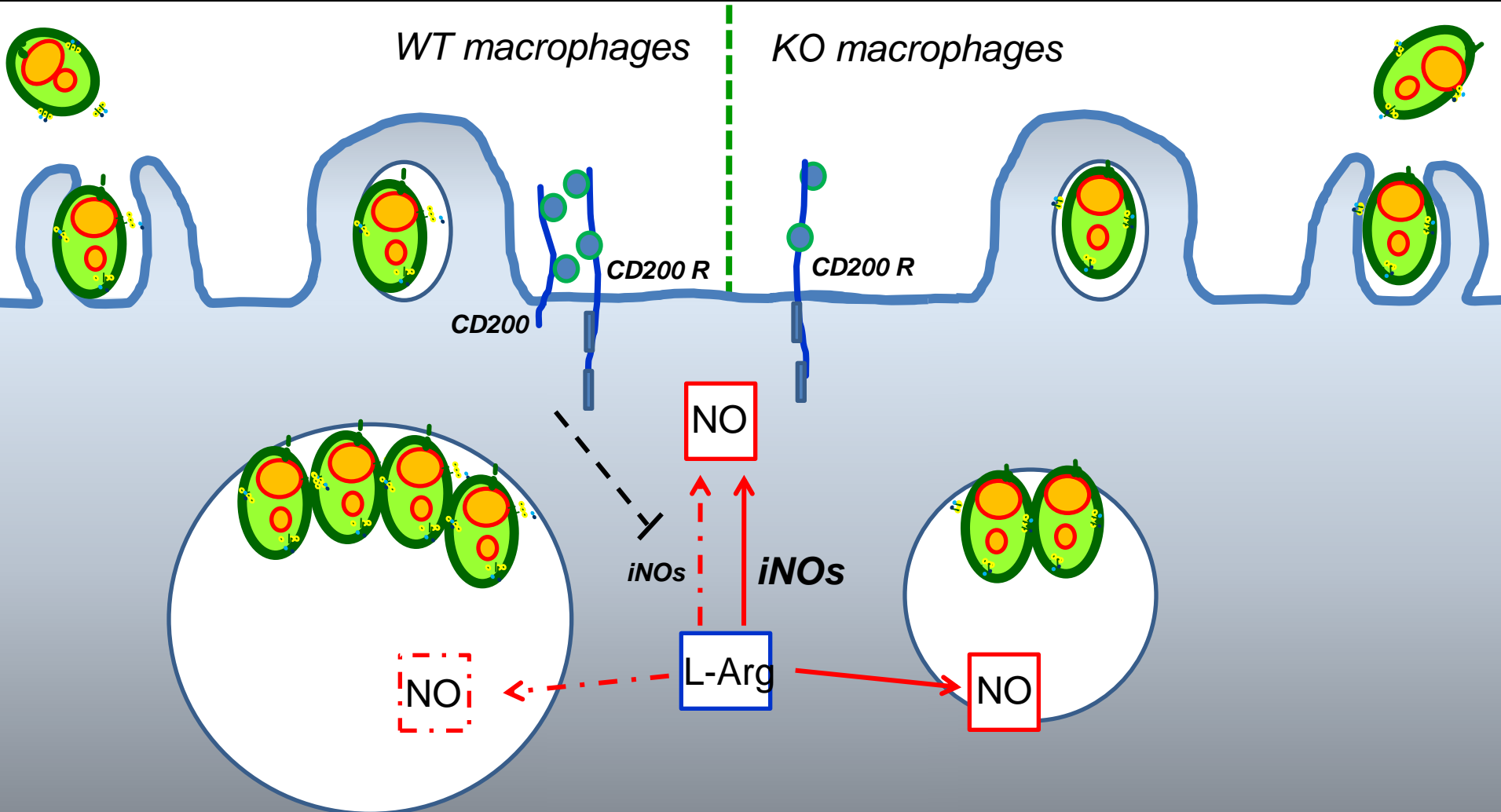
*L. (L.) amazonensis* induces CD200 and inhibits iNOS-NO production - important response to kill parasites



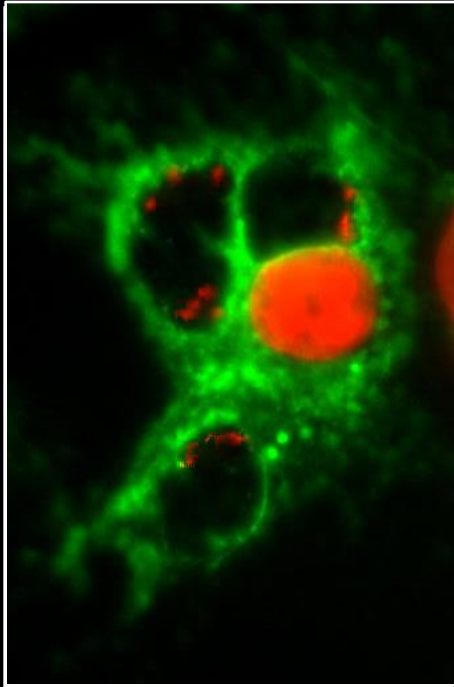
*L. (L.) amazonensis* induces CD200 and inhibits iNOS-NO production - important response to kill parasites



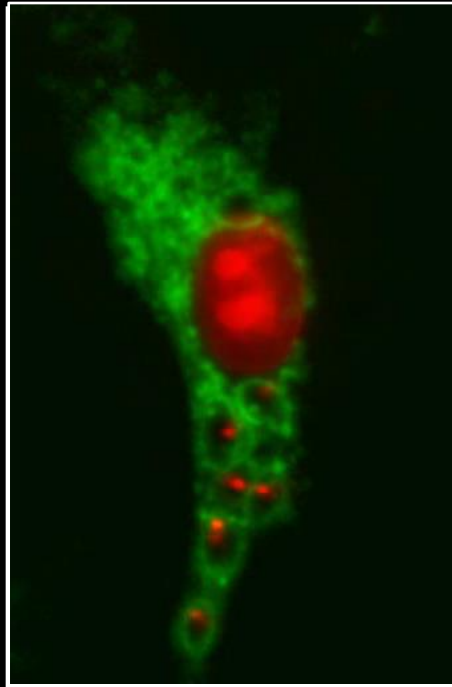
*L. (L.) amazonensis* induces CD200 and inhibits macrophage iNOS - important response to kill parasites



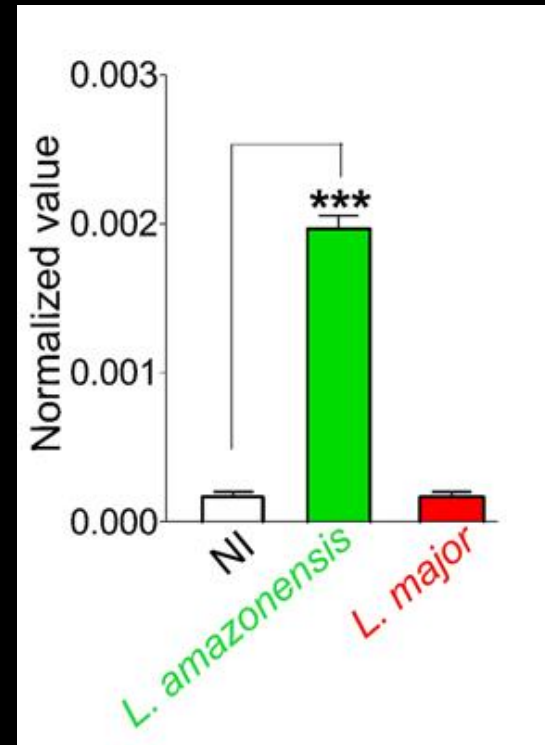
# Why *L. (L.) amazonensis* is more virulent than *L. (L.) major*?



*L. (L.) amazonensis*

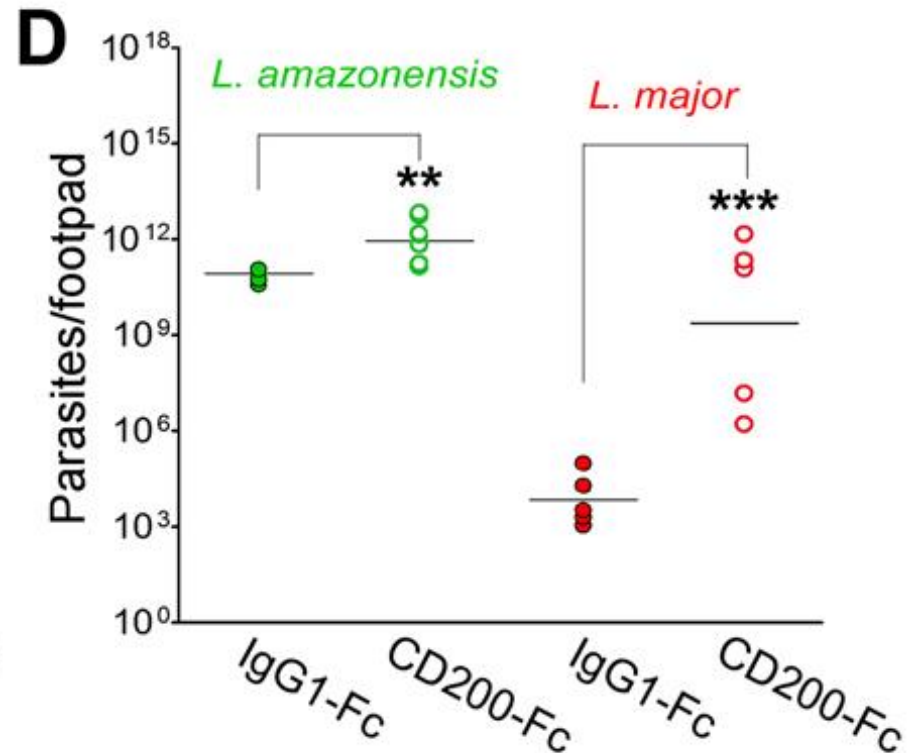
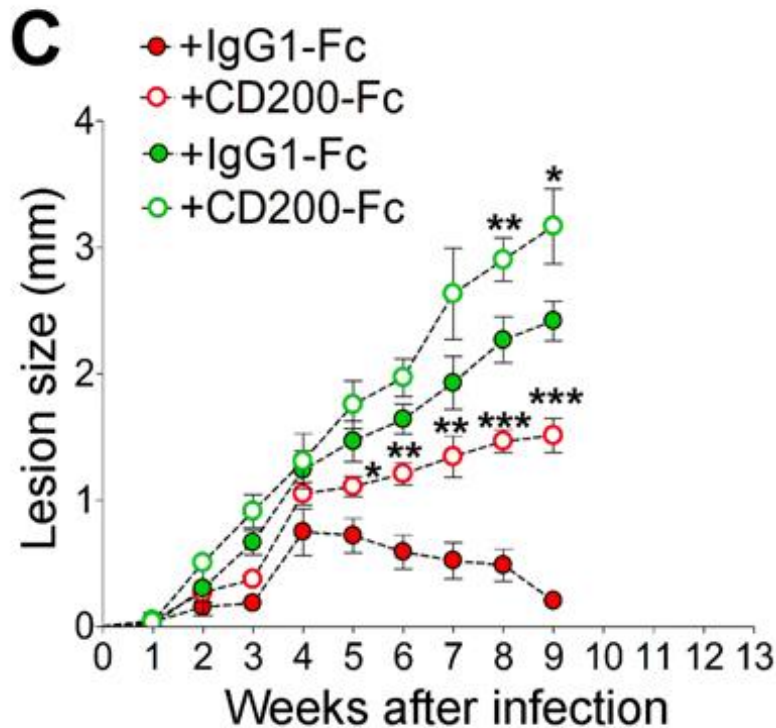


*L. (L.) major*

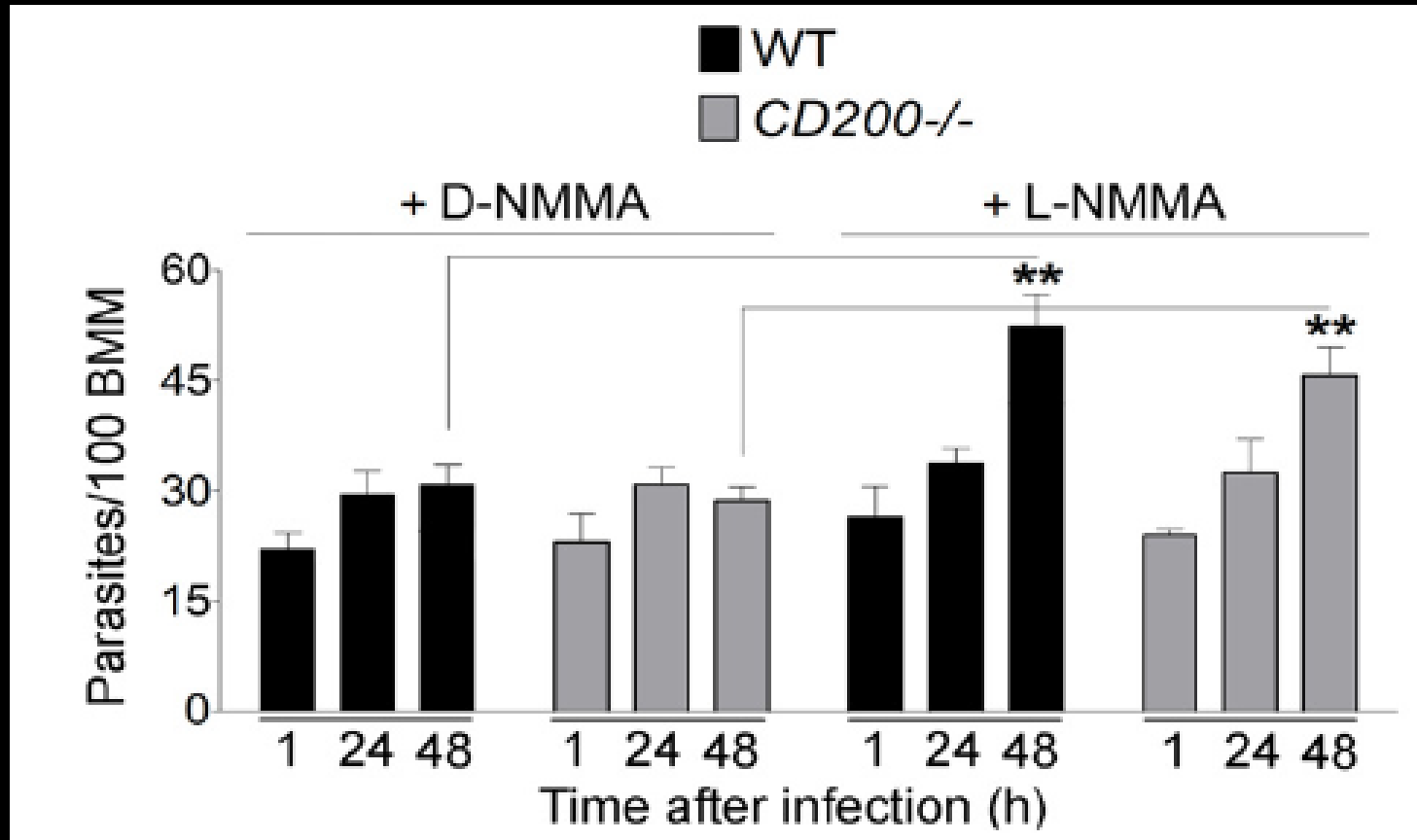


*L. (L.) major* fails to induce CD200 in macrophages

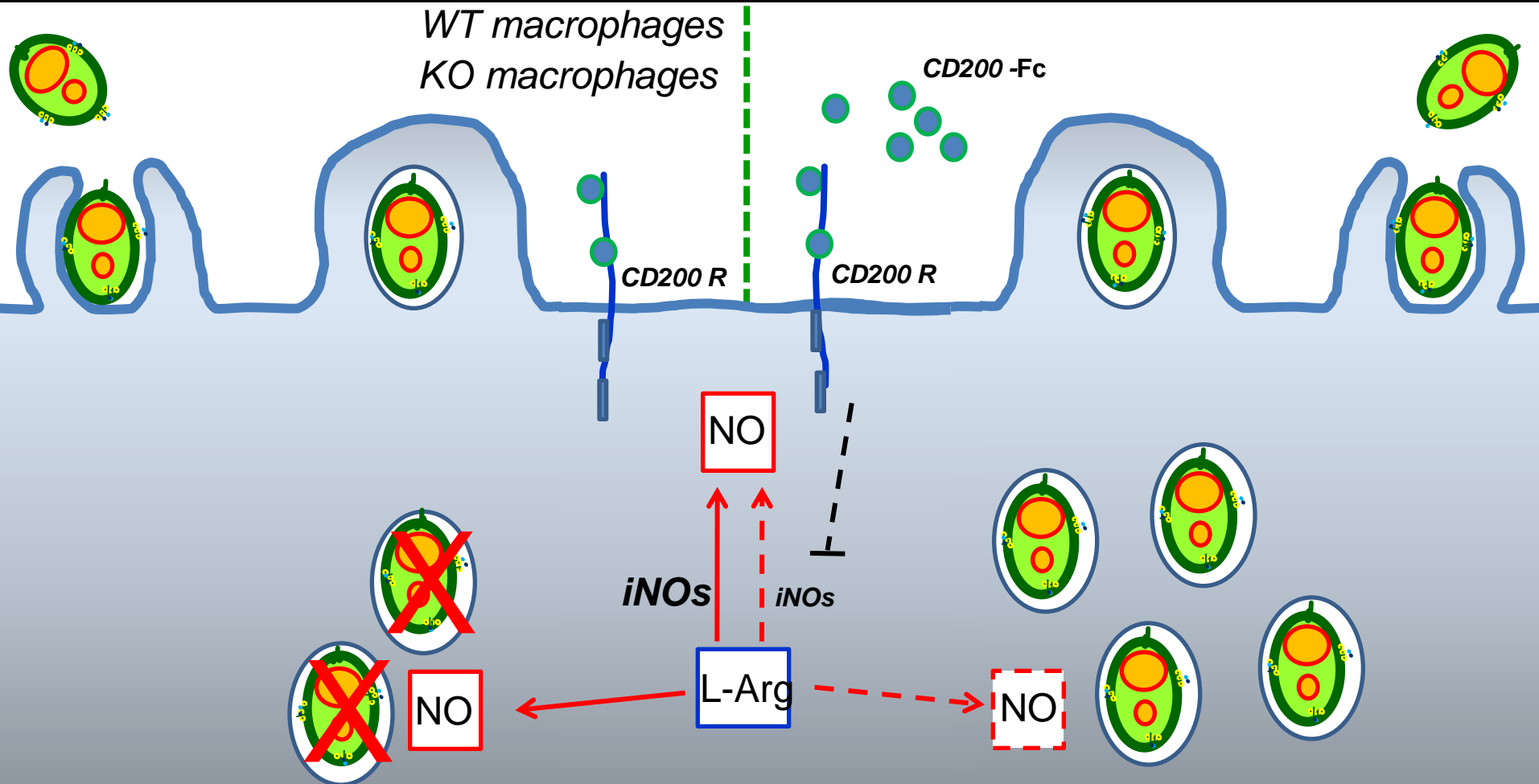
CD200 transforms the mild *L. (L.) major* infection into a virulent pattern typical of *L. (L.) amazonensis*



# Inhibition of iNOS recover infectivity of *L. (L.) major* in macrophages

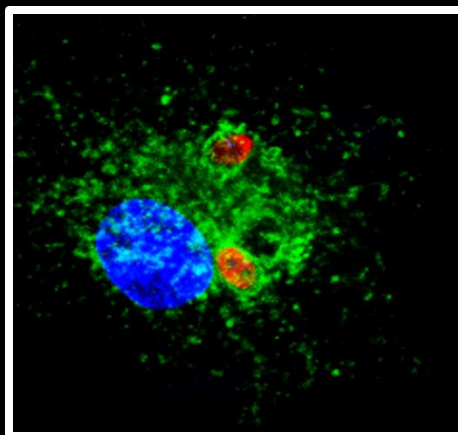


# *L. (L.) major* fails to induce CD200 in macrophages

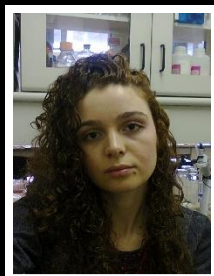




# Cortez Lab

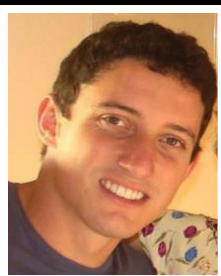


Lab manager  
2012



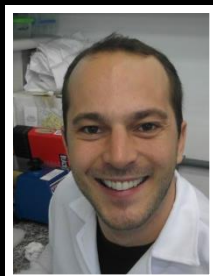
Juliana  
ávilla

Doutorando  
2013



Ismael  
Sauter

Mestrando  
2012



Albert  
Bressan

Mestrando  
2013



Lina  
Borda

Mestrando  
2013



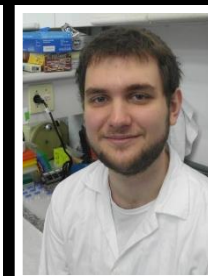
Natalia  
Soares

Mestrando  
2014



Natalia  
Arango

IC  
2012



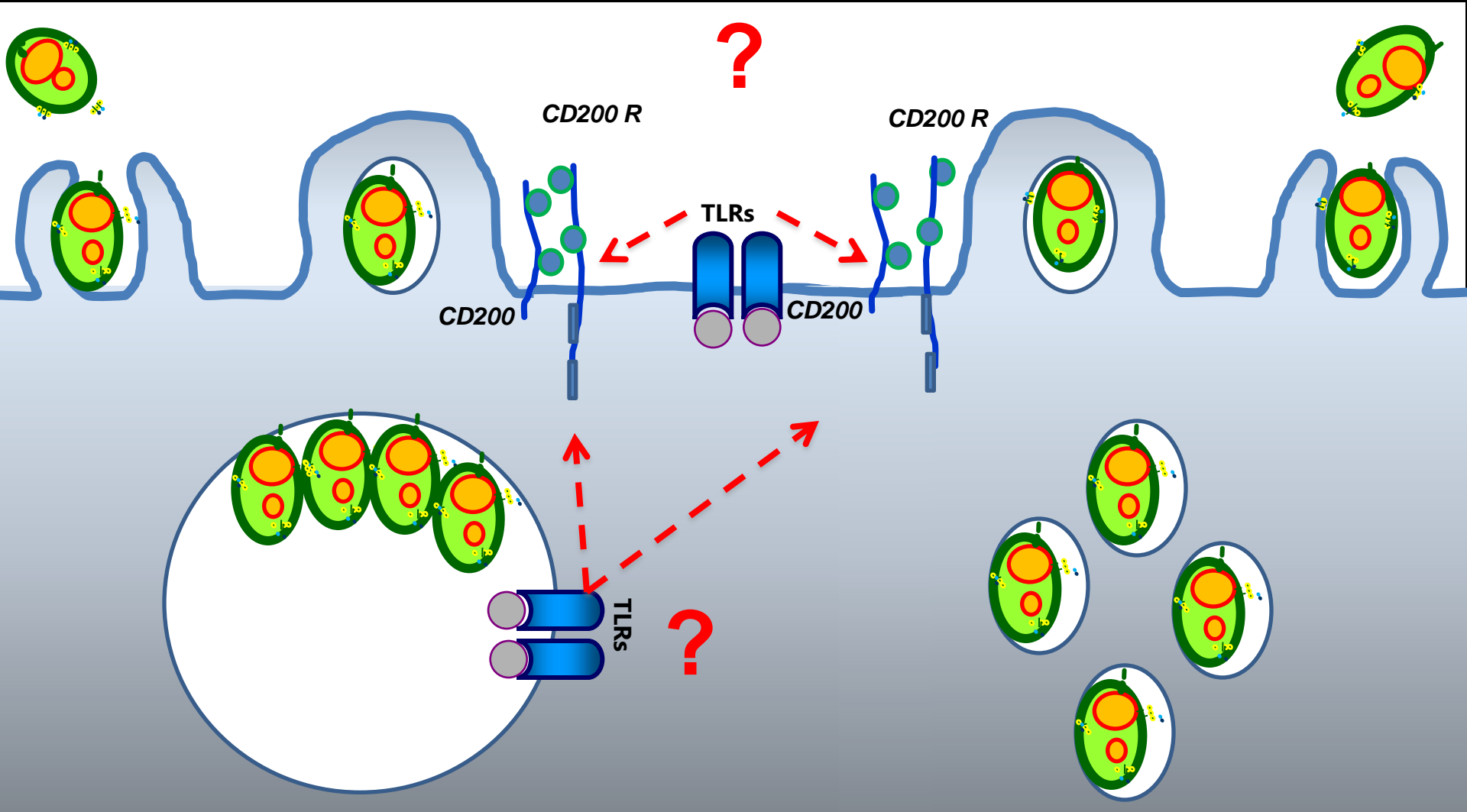
Andreas  
Duva

IC  
2013



Viviane C.  
de Lima

# Is CD200 induction dependent of TLR ?



# Is CD200 induction in macrophages infected by *Leishmania* TLR-dependent ?



Ismael Sauter

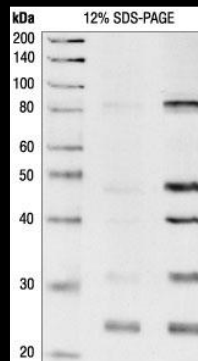
C57BL/6 WT



C57BL/6 MyD88<sup>-/-</sup>



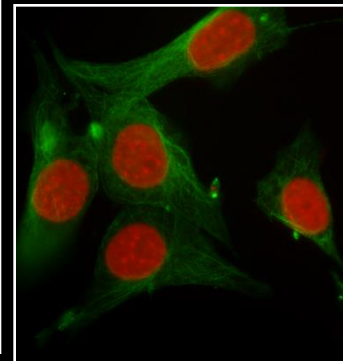
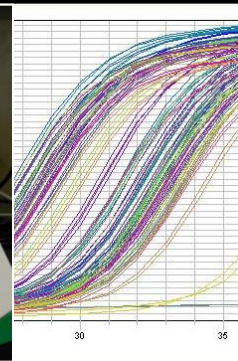
C57BL/6 TRIF<sup>-/-</sup>



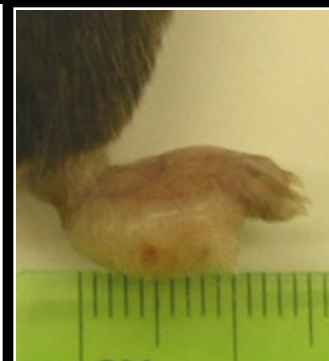
WB



qPCR

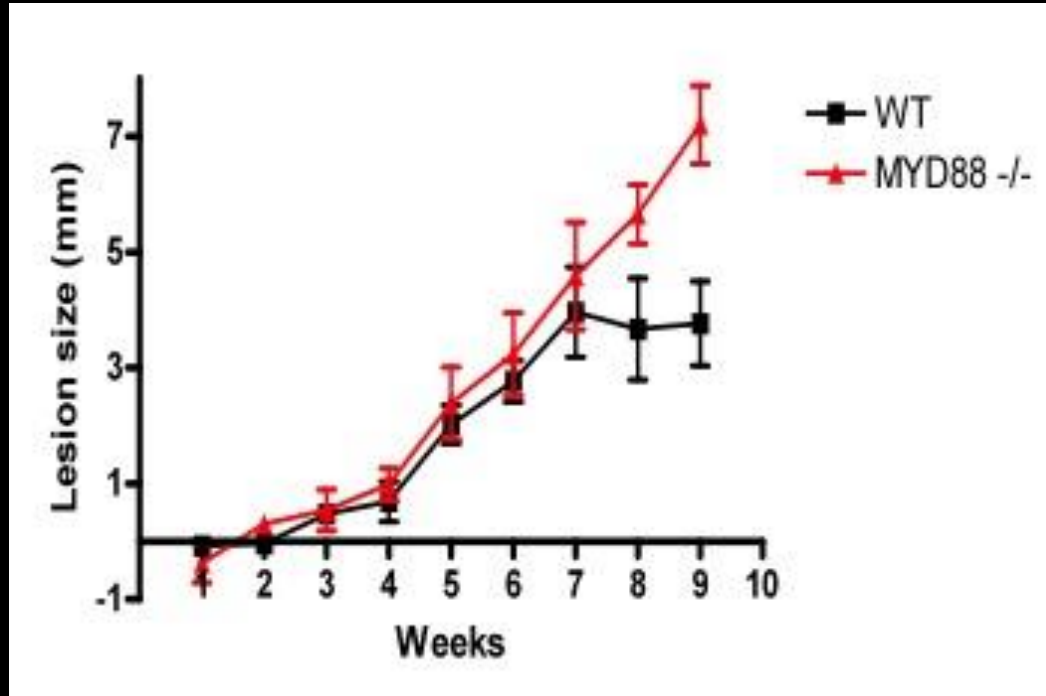


*in vitro* infection

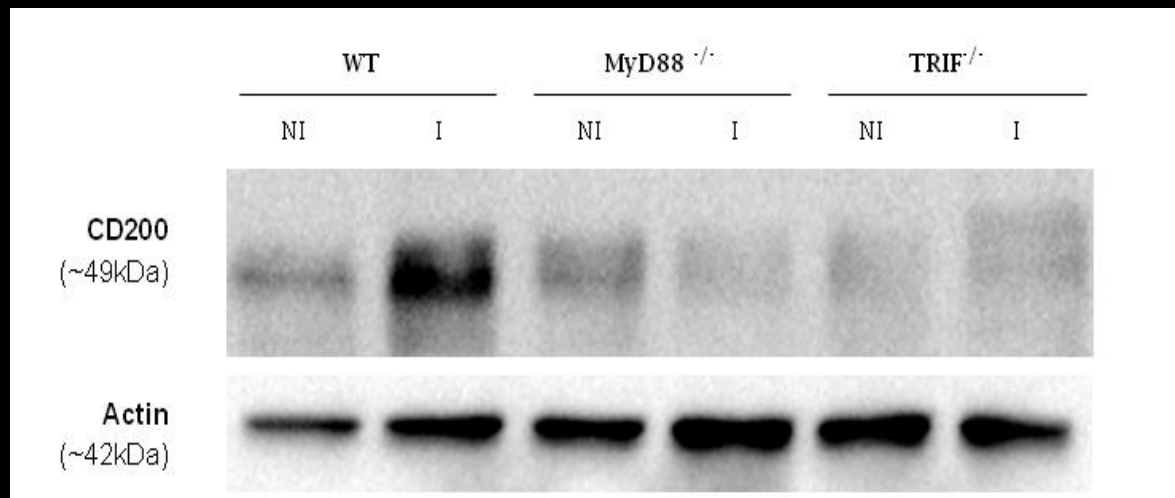
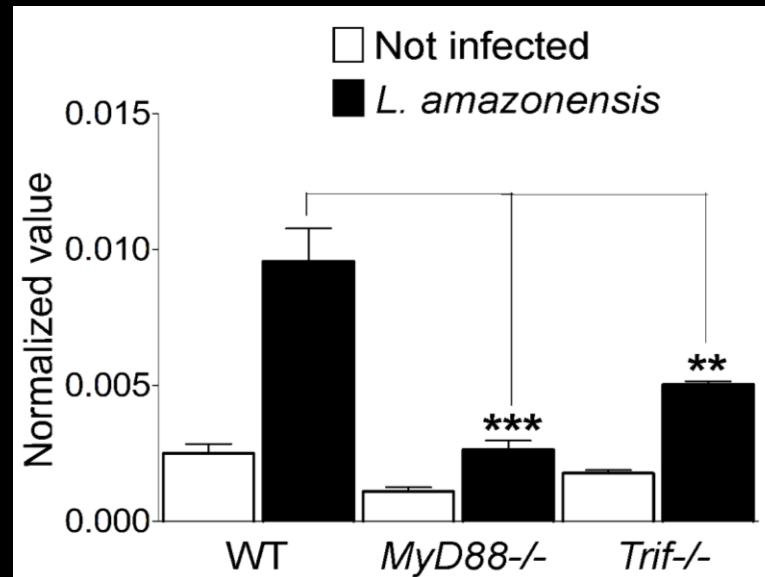


*in vivo*

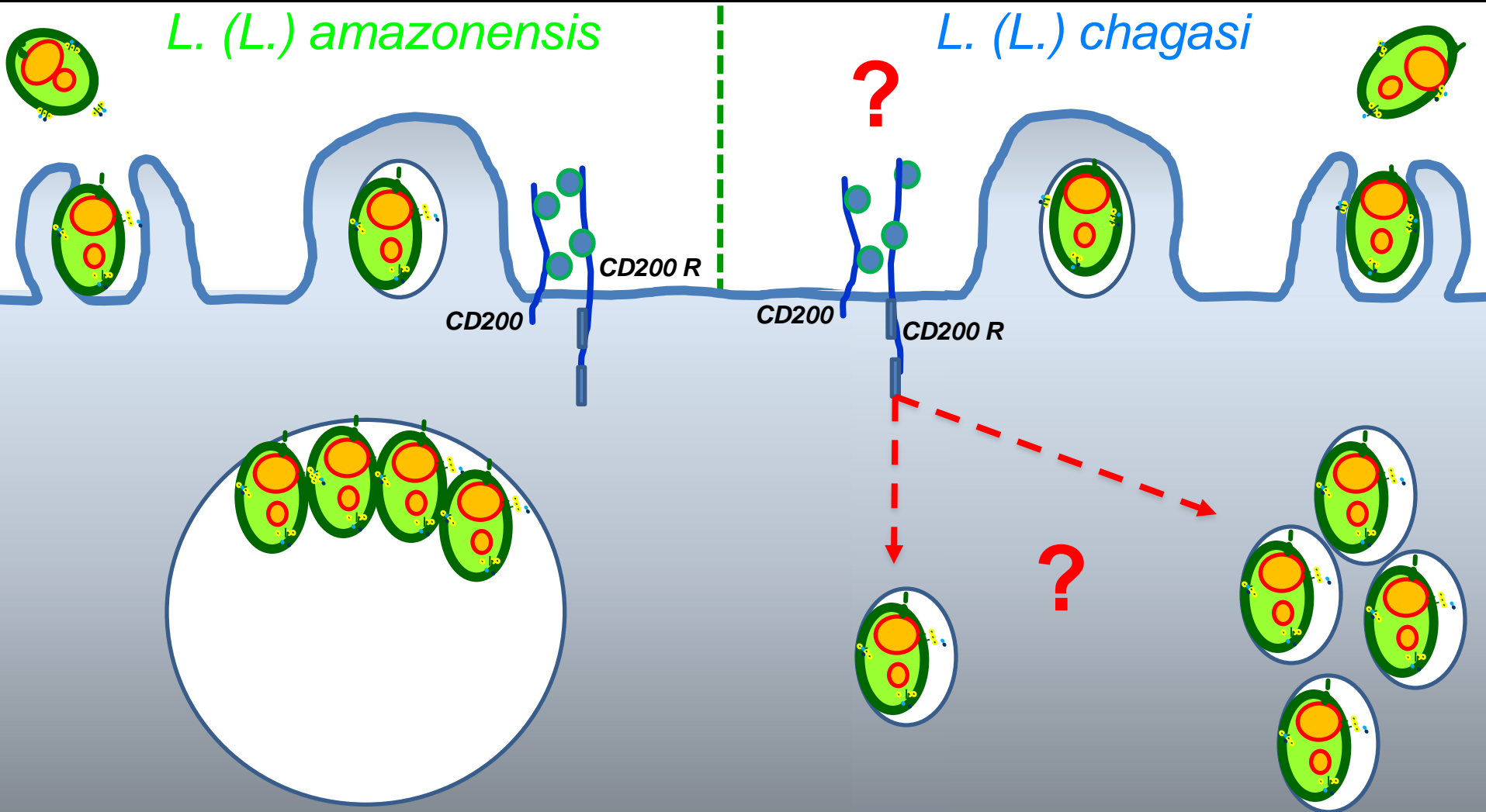
## *L.(L.) amazonensis* infection in MyD88 $-/-$ mice



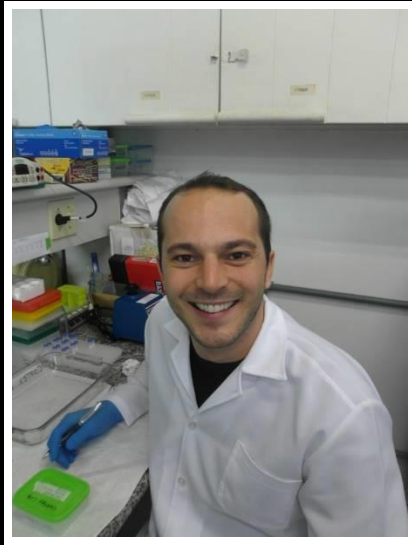
*L. amazonensis* induces CD200 in bone marrow macrophages (BMM) of WT mice but not in deficient mice



*L. (L.) amazonensis* vs *L. (L.) chagasi*  
Role of CD200 in different *Leishmanias*



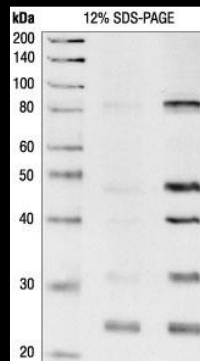
# Is CD200 induction important for *Leishmania* (*L.*) *chagasi* infection?



Albert Bressan



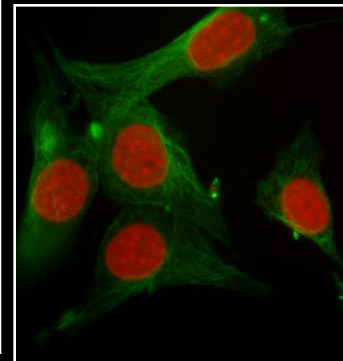
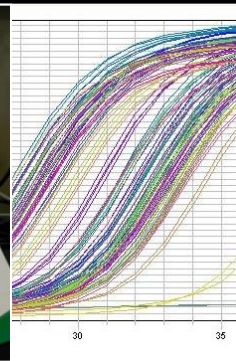
C57BL/6 WT



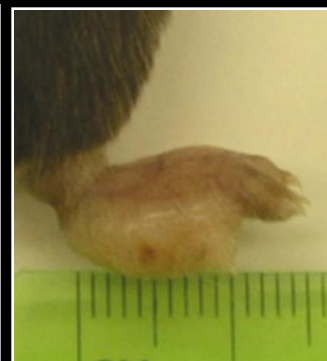
WB



PCR em tempo real

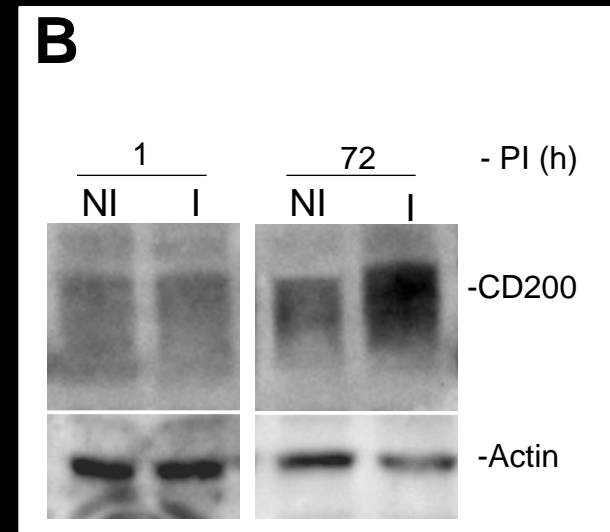
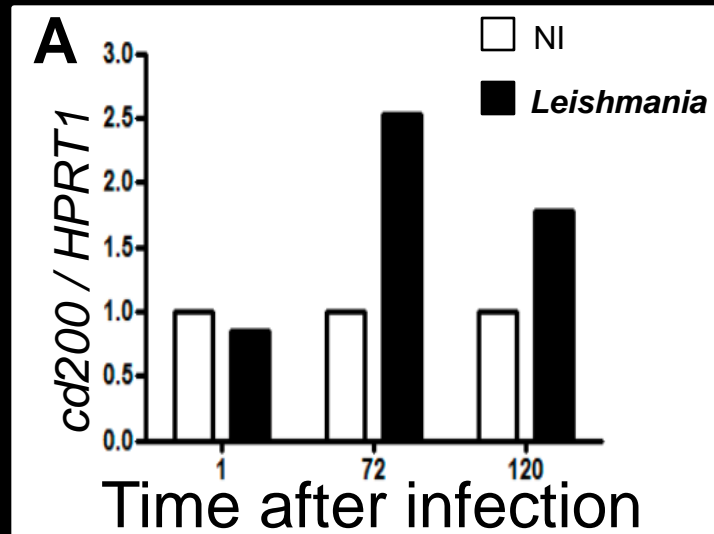


infecção *in vitro*

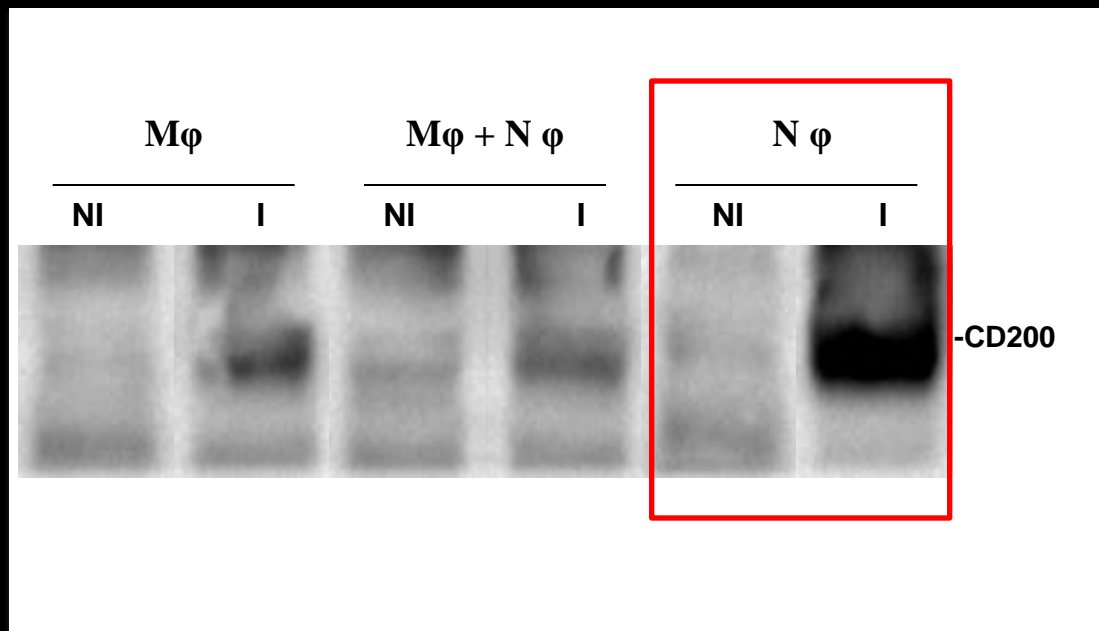


*in vivo*

*L. (L.) chagasi* induces CD200 in bone marrow macrophages (BMM) at later time-points of infection



*L. (L.) chagasi* induces CD200 in neutrophils of C57BL/6 mice



Role of different cells in *Leishmania* infection

# *L. (L.) chagasi* induces CD200 in splenocytes of infected mice

NI

$1 \times 10^7$  stationary phase forms of  
*L. (L.) infantum chagasi*



NI

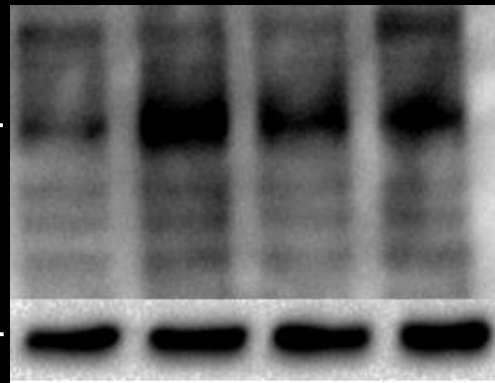
1

2

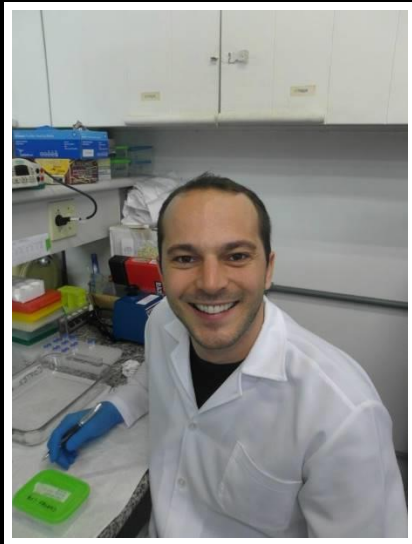
3

CD200-

Actin-



# Is CD200 induction important for *Leishmania* (*L.*) *chagasi* infection?



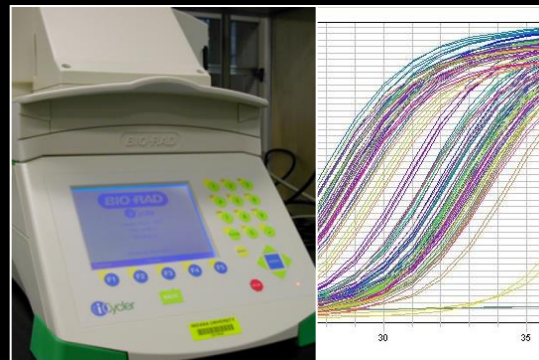
Albert  
Bressan



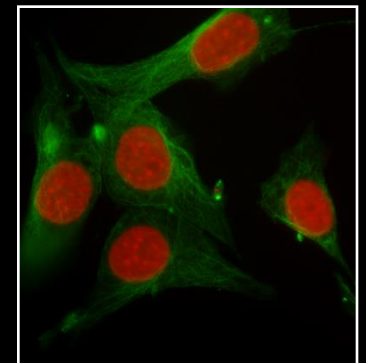
Dra. Clara L. Barbieri



Biotério da Parasitologia  
UNIFESP-EPM



PCR em tempo real



**infecção *in vitro***

# Cutaneous leishmaniasis species:

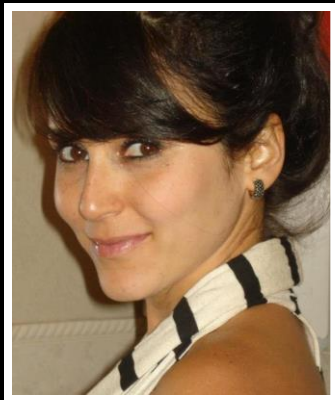


## Collaborators at Fiocruz de Bahia- Brazil

# Exposure of Phosphatidylserine on *Leishmania amazonensis* Isolates Is Associated with Diffuse Cutaneous Leishmaniasis and Parasite Infectivity

Jaqueline França-Costa<sup>1,2\*</sup>, João Luiz Mendes Wanderley<sup>3,4\*</sup>, Poliana Deolindo<sup>4,5</sup>, Jessica B. Zarattini<sup>4,6</sup>, Jackson Costa<sup>1</sup>, Lynn Soong<sup>7</sup>, Marcello André Barcinski<sup>4,5</sup>, Aldina Barral<sup>1,2</sup>, Valeria M. Borges<sup>1,2\*</sup>

Jaqueline França-Costa



Marcello A. Barcinski



Valeria M. Borges



# Exposure of Phosphatidylserine on *Leishmania amazonensis* Isolates Is Associated with Diffuse Cutaneous Leishmaniasis and Parasite Infectivity

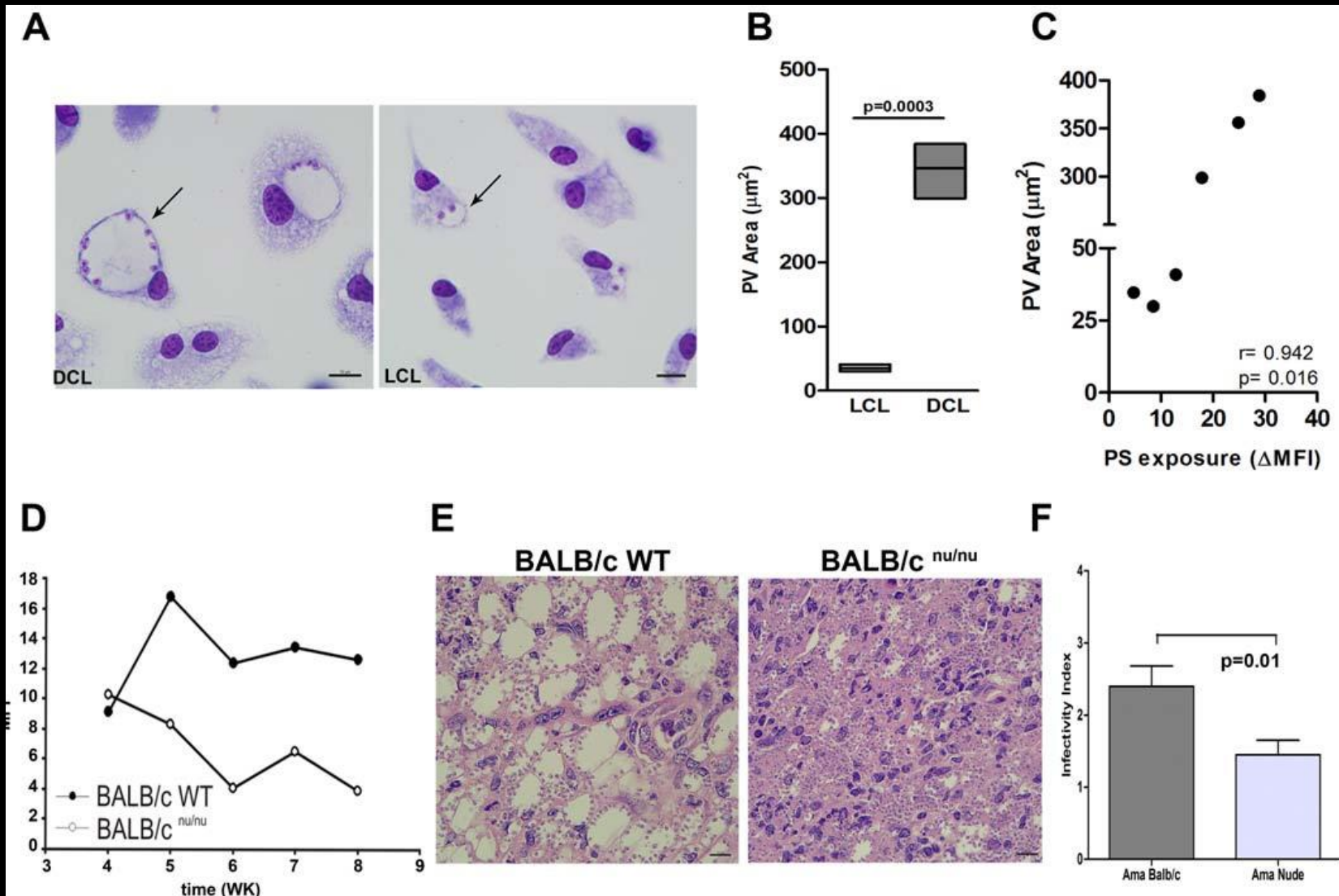
Jaqueline França-Costa<sup>1,2\*</sup>, João Luiz Mendes Wanderley<sup>3,4\*</sup>, Poliana Deolindo<sup>4,5</sup>, Jessica B. Zarattini<sup>4,6</sup>, Jackson Costa<sup>1</sup>, Lynn Soong<sup>7</sup>, Marcello André Barcinski<sup>4,5</sup>, Aldina Barral<sup>1,2</sup>, Valeria M. Borges<sup>1,2\*</sup>

Table 1. *Leishmania amazonensis* isolates used in this study.

Number	Strain	International Code (*)	Origin	Pathology
1	BA 106	MHOM/BR/1986/BA106	BA	DCL
2	BA 199	MHOM/BR/1989/BA199	MA	DCL
3	BA 276	MHOM/BR/1989/BA276	MA	DCL
4	BA 336	MHOM/BR/1990/BA336	MA	DCL
5	BA 700	MHOM/BR/1997/BA700	MA	DCL
6	BA 760	MHOM/BR/1999/BA760	MA	DCL
7	BA 820	MHOM/BR/2002/BA820	MA	DCL
8	BA 69	MHOM/BR/1985/BA 69	BA	LCL
9	BA 73	MHOM/BR/1985/BA 73	BA	LCL
10	BA115	MHOM/BR/1987/BA115	BA	LCL
11	BA125	MHOM/BR/1987/BA125	BA	LCL
12	BA113	MHOM/BR/1987/BA113	BA	LCL
13	M2269	MHOM/BR/1973/M2269	PA	LCL
14	LV79	MPRO/BR/72M 1841-LV-79	BR	LCL

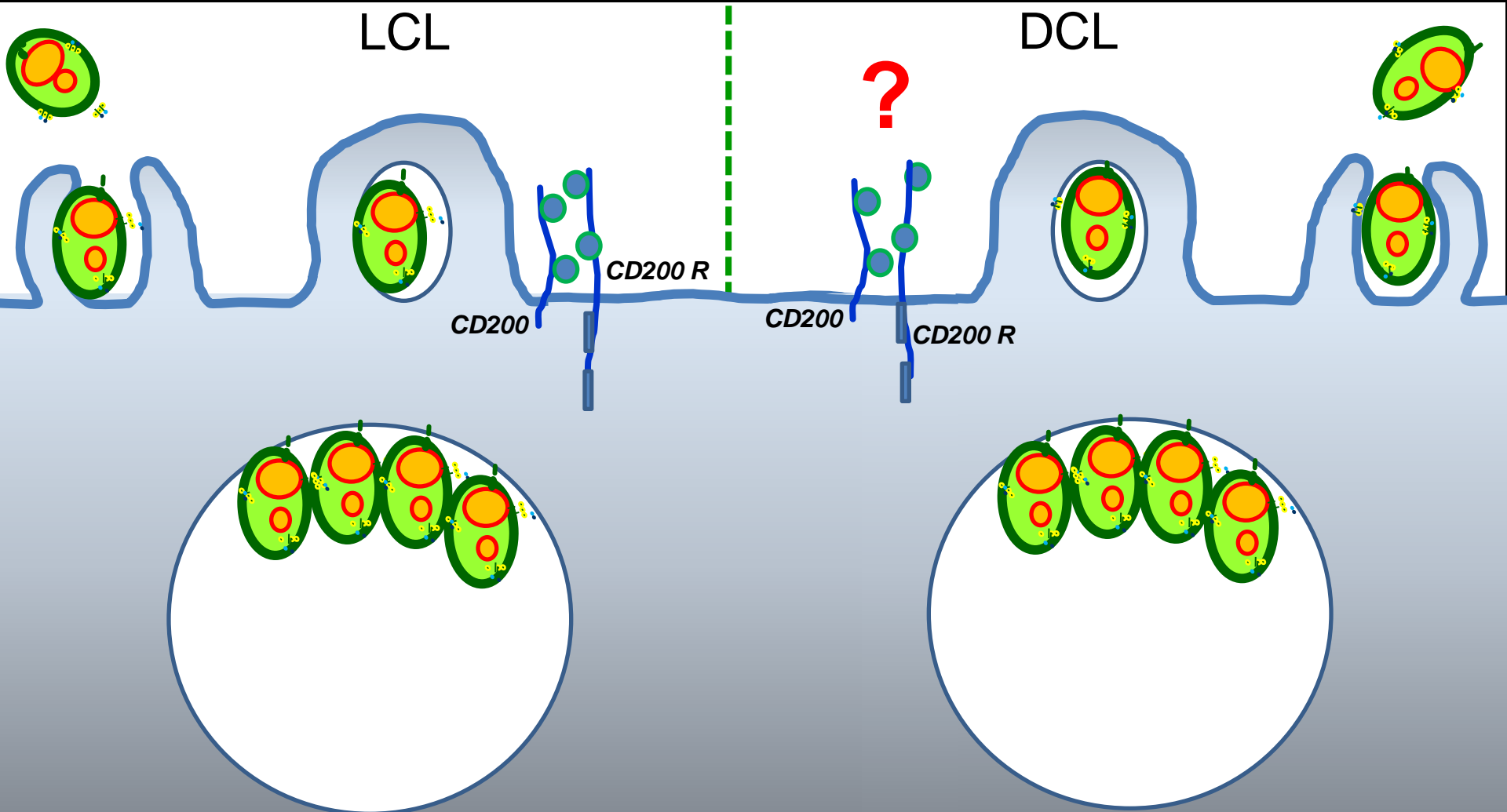
# *L. (L.) amazonensis* vs *L. (L.) amazonensis*

## LCL vs DCL



# *L. (L.) amazonensis* vs *L. (L.) amazonensis*

Role of CD200 in isolates from patients with LCL and DCL



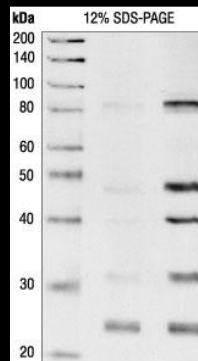
*L. (L.) amazonensis* vs *L. (L.) amazonensis*  
Role of CD200 in isolates from patients with LCL and DCL



Lina Borda



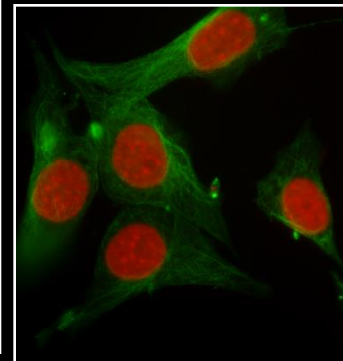
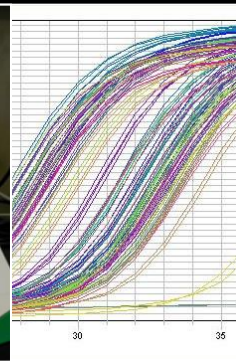
C57BL/6 WT



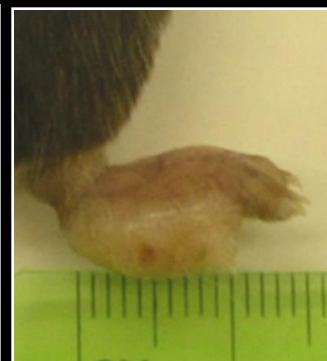
WB



qPCR

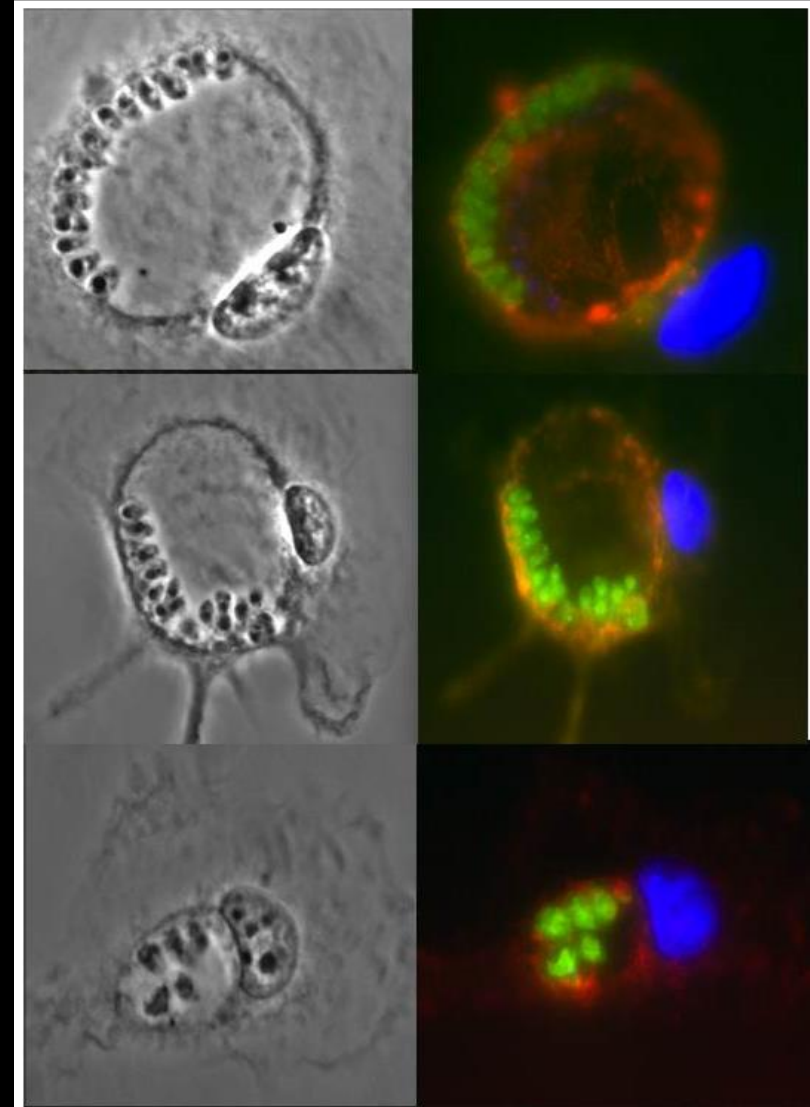
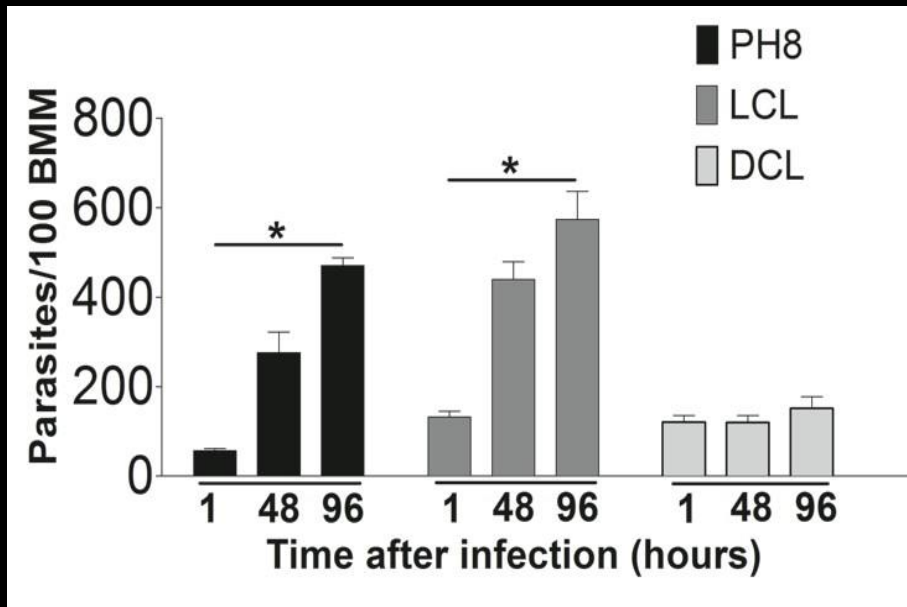


*in vitro* infection

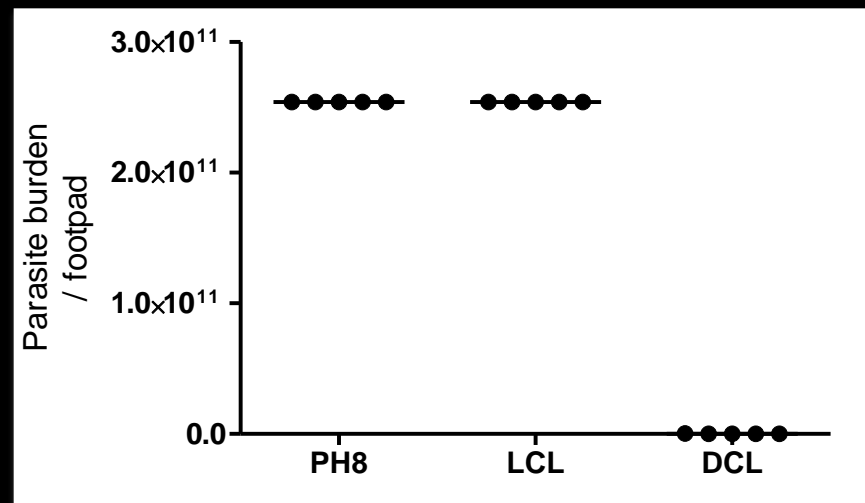
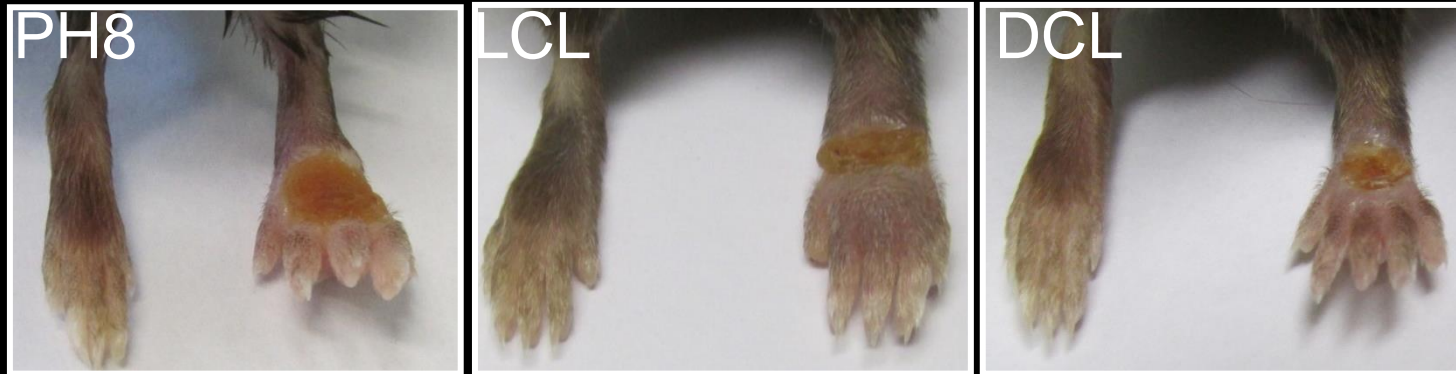


*in vivo*

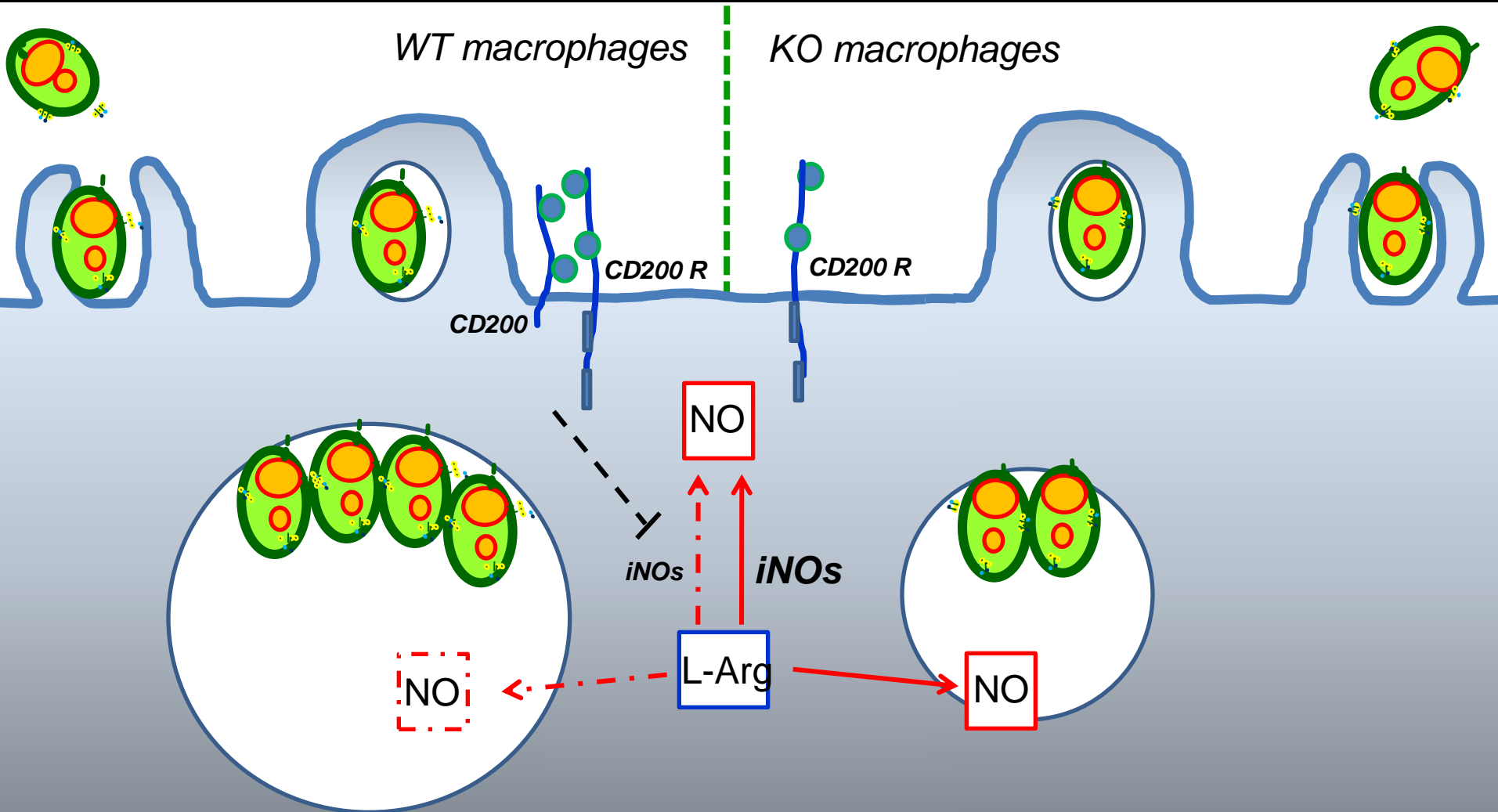
*L. (L.) amazonensis* isolates associated with diffuse cutaneous leishmaniasis (DCL) fails to proliferate in macrophage of C57BL/6 mice



*L. (L.) amazonensis* isolates associated with diffuse cutaneous leishmaniasis (DCL) is controlled in C57BL/6 mice infection

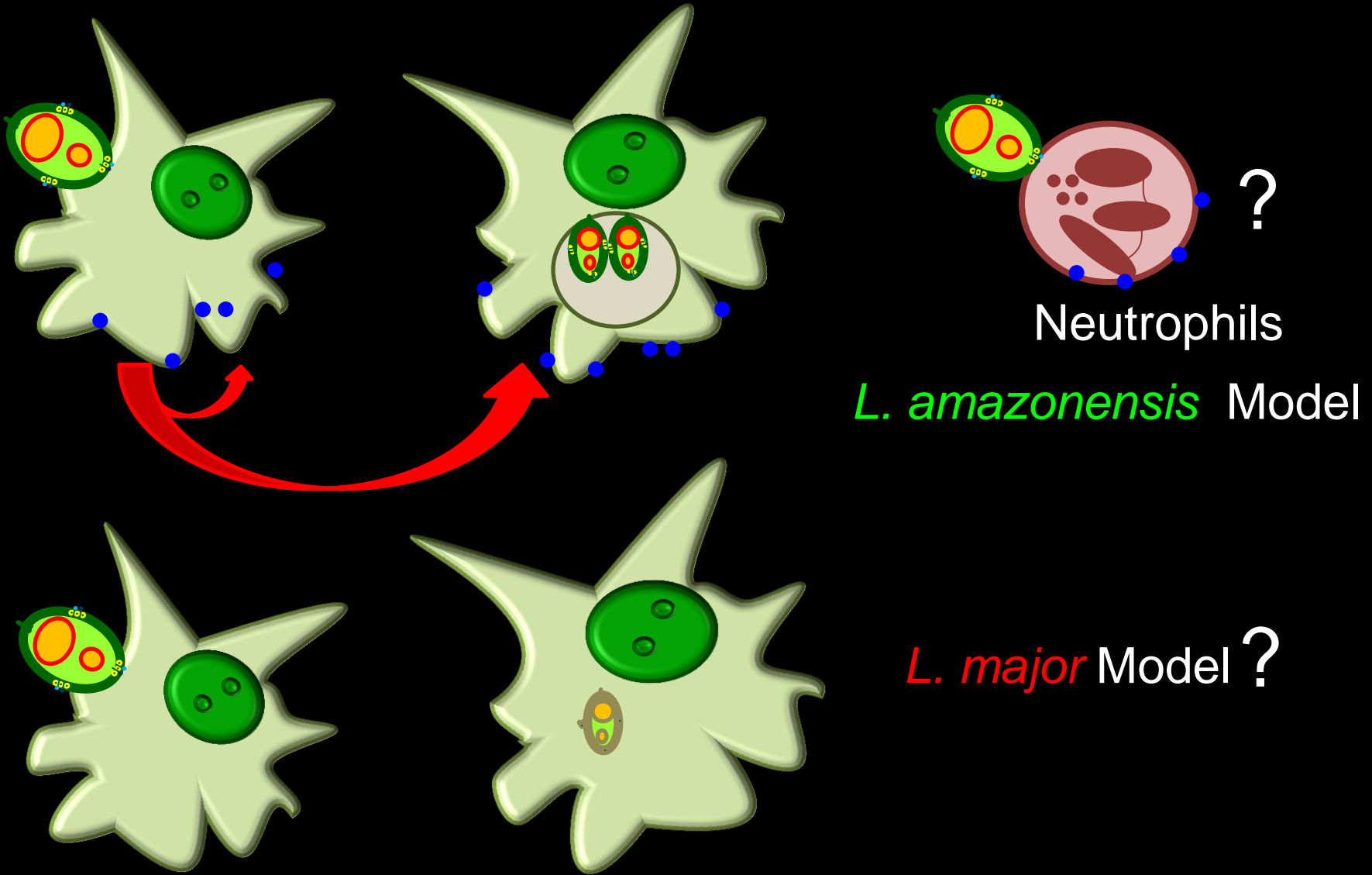


# Role of CD200-CD200R in *Leishmania* infection



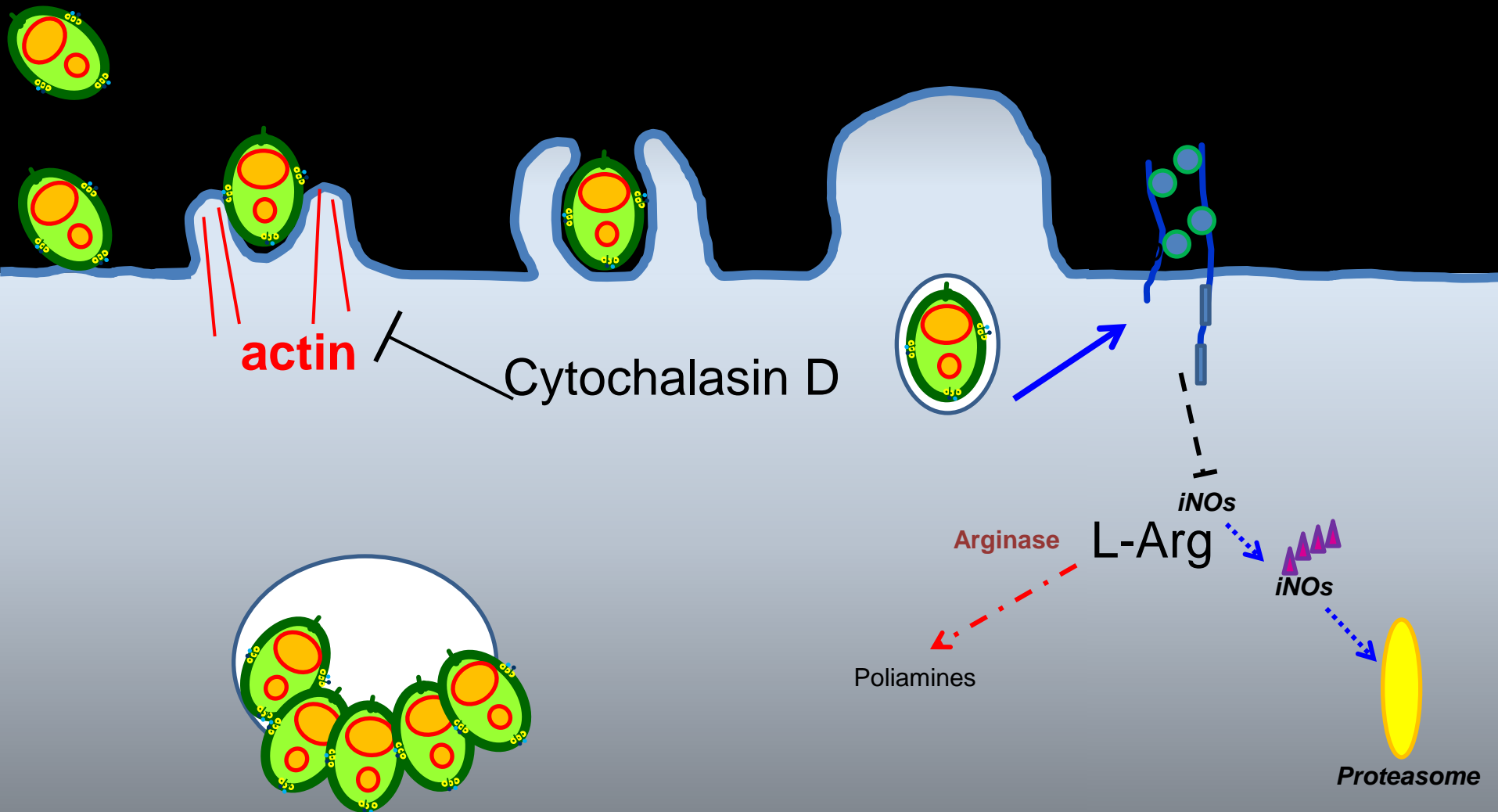
# Future experiments:

- To see the involvement of others cells in the CD200 signal.

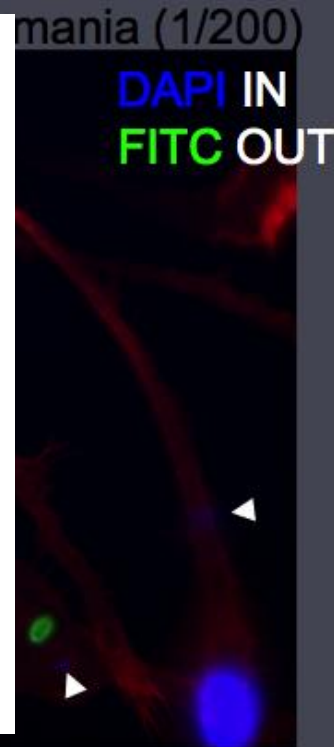
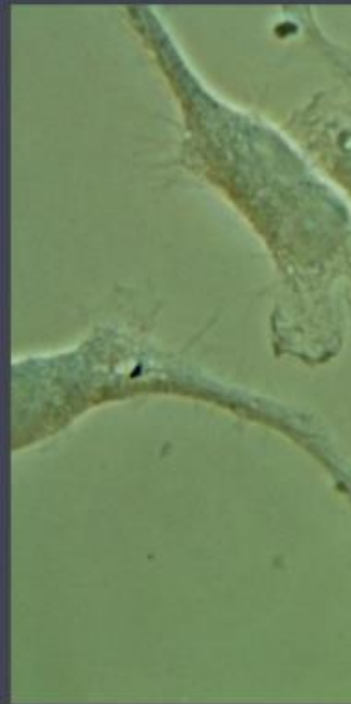
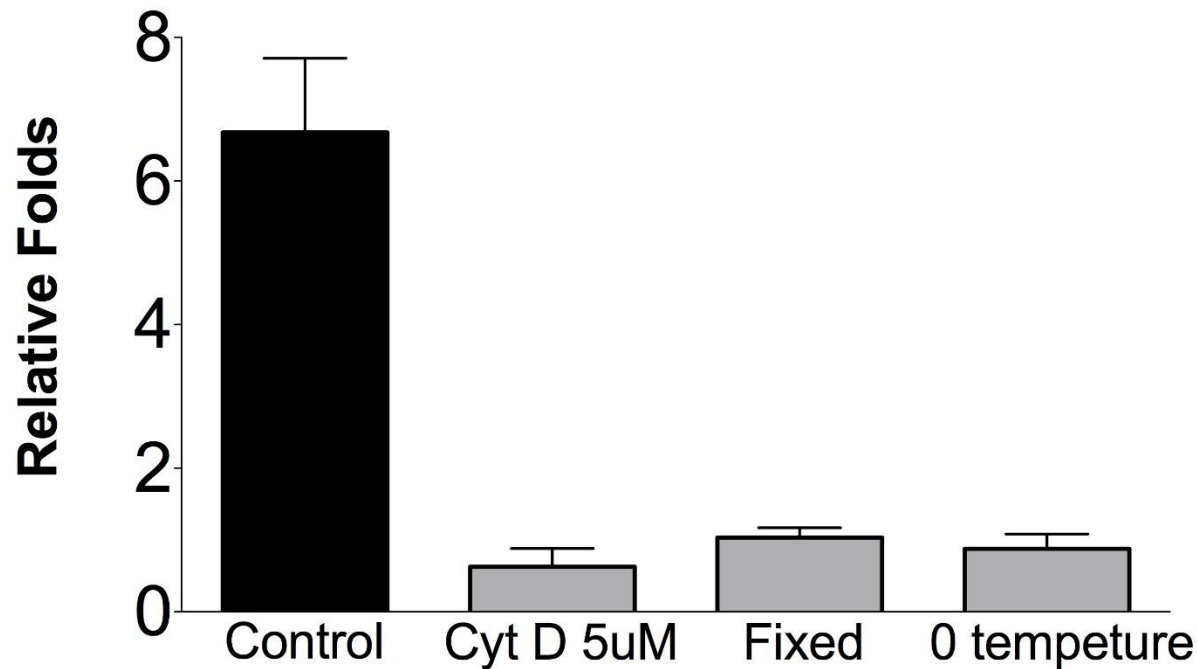


# Future experiments:

-To see where is activated the CD200 signal in macrophages infected with *Leishmania*.



# Induction of CD200 by *Leishmania* in macrophages treated previously with actin polymerization



# Acknowledgments:

Brazil:

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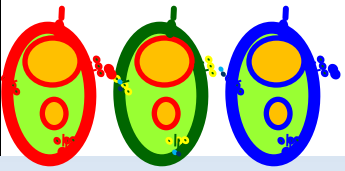
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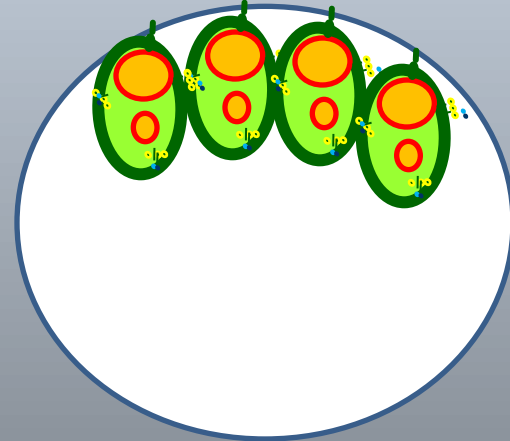
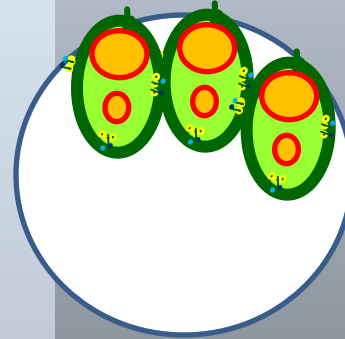
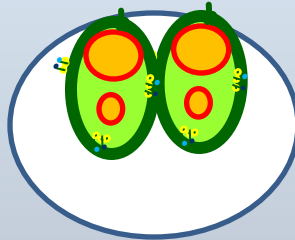
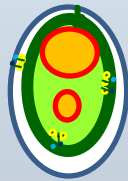
# Sao Paulo, Brazil



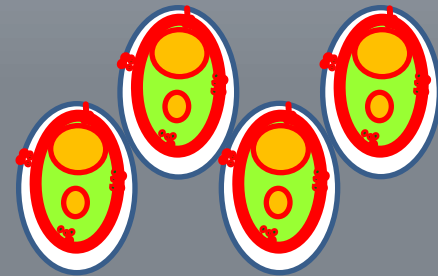
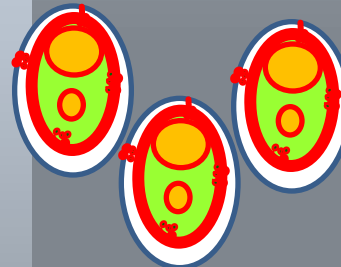
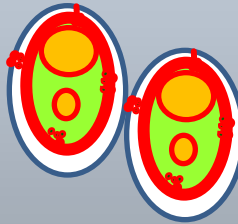
# Introdução: Ensaios de infecção in vitro



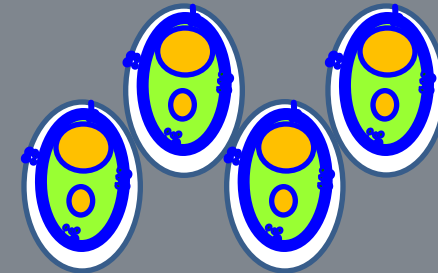
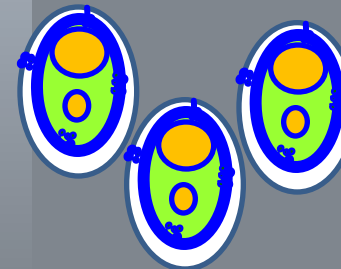
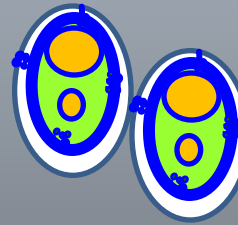
*L. amazonensis*

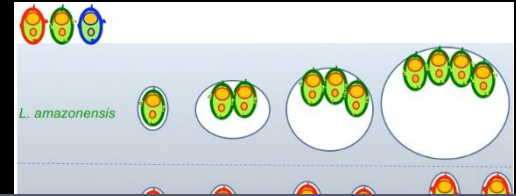


*L. major*

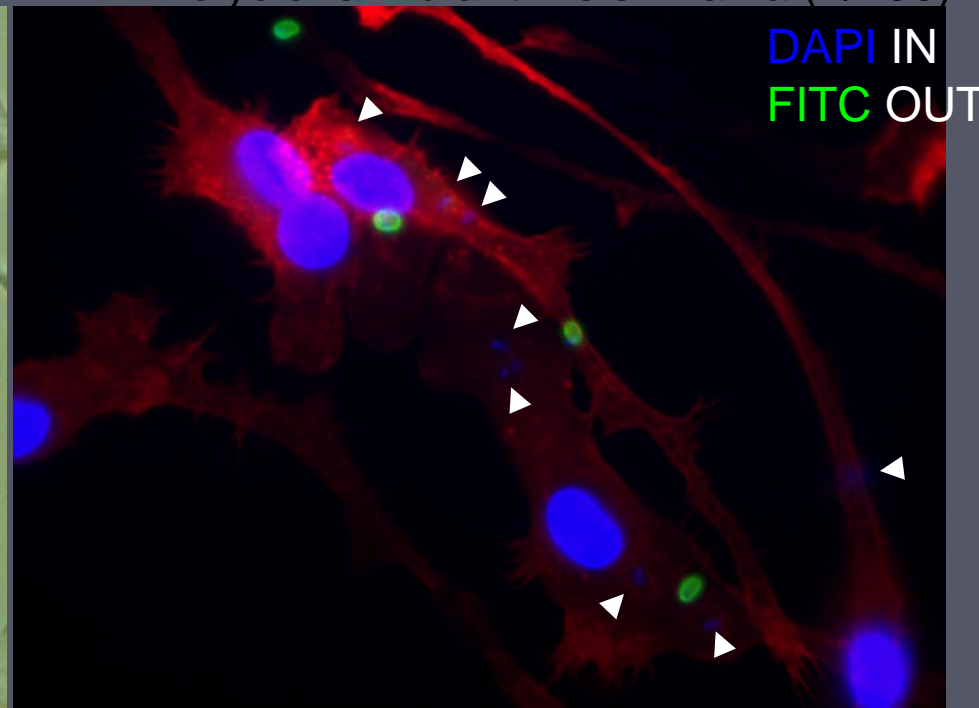
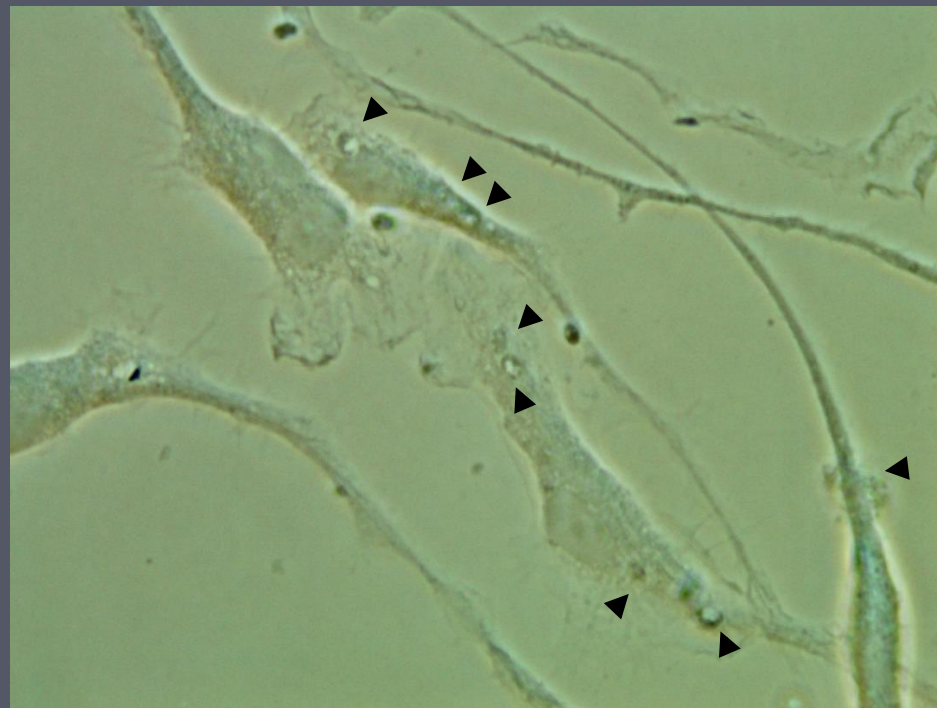


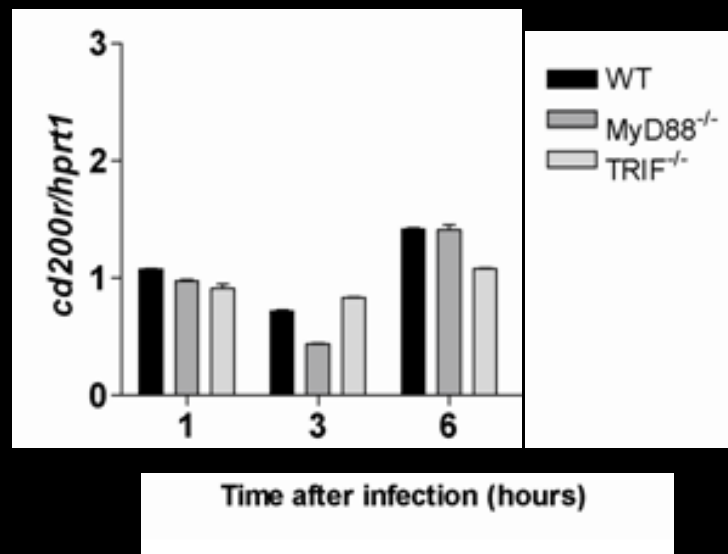
*L. chagasi*





Polyclonal Ab anti-Leishmania (1/200)



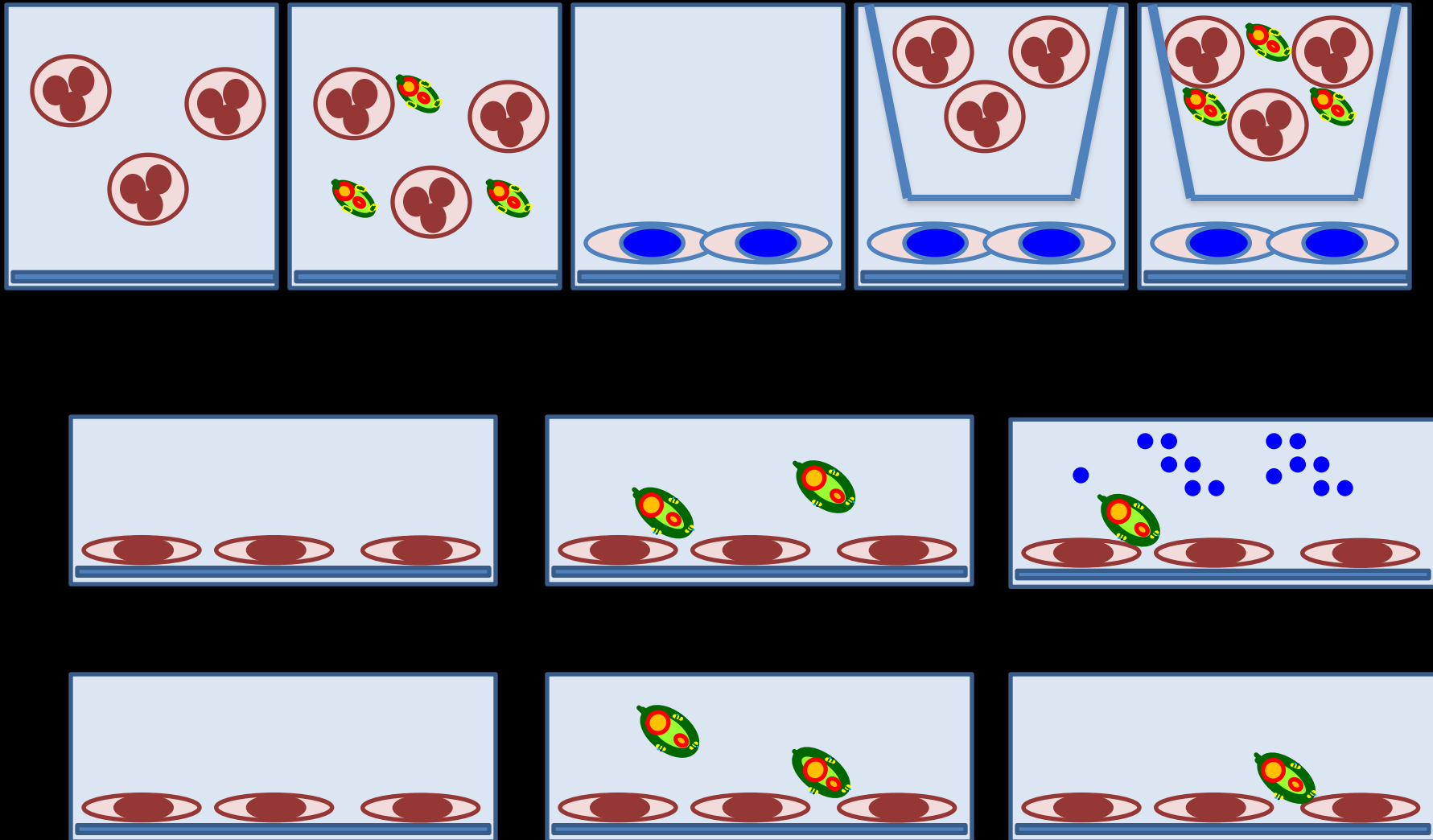


# Schematic procedure:




Wash out

CD200: 4 $\mu$ g/mL

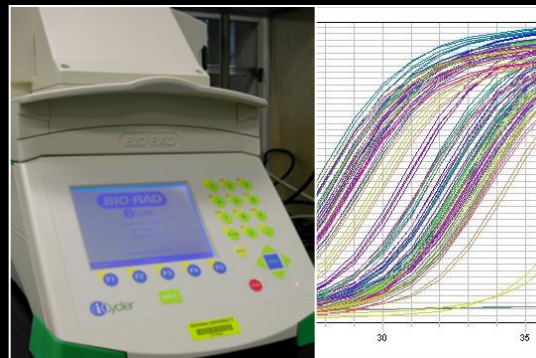
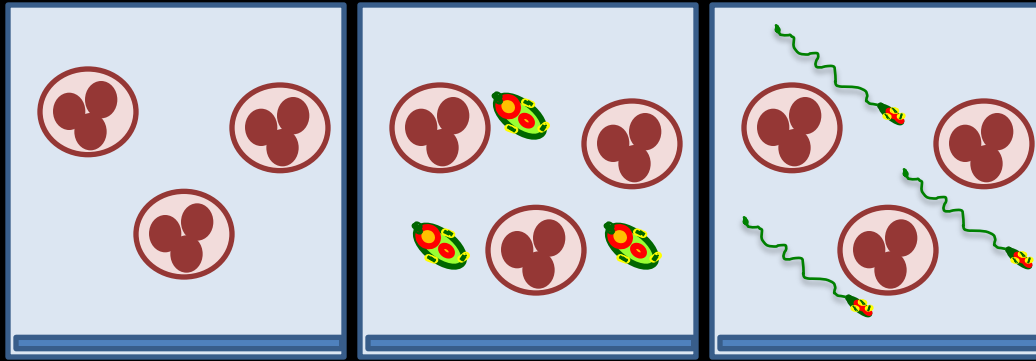


# Schematic procedure:

 amastigotes

 Neutrophils

 metacyclic



PCR em tempo real

## New schematic procedure:

1 day: Infection  $1 \times 10^6$  parasites/footpad +  $10^{-10}$  µg CD205Fc

3 day: + 10  $\mu$ g CD200-Fc

4 day: + 10  $\mu$ g CD200-Fc

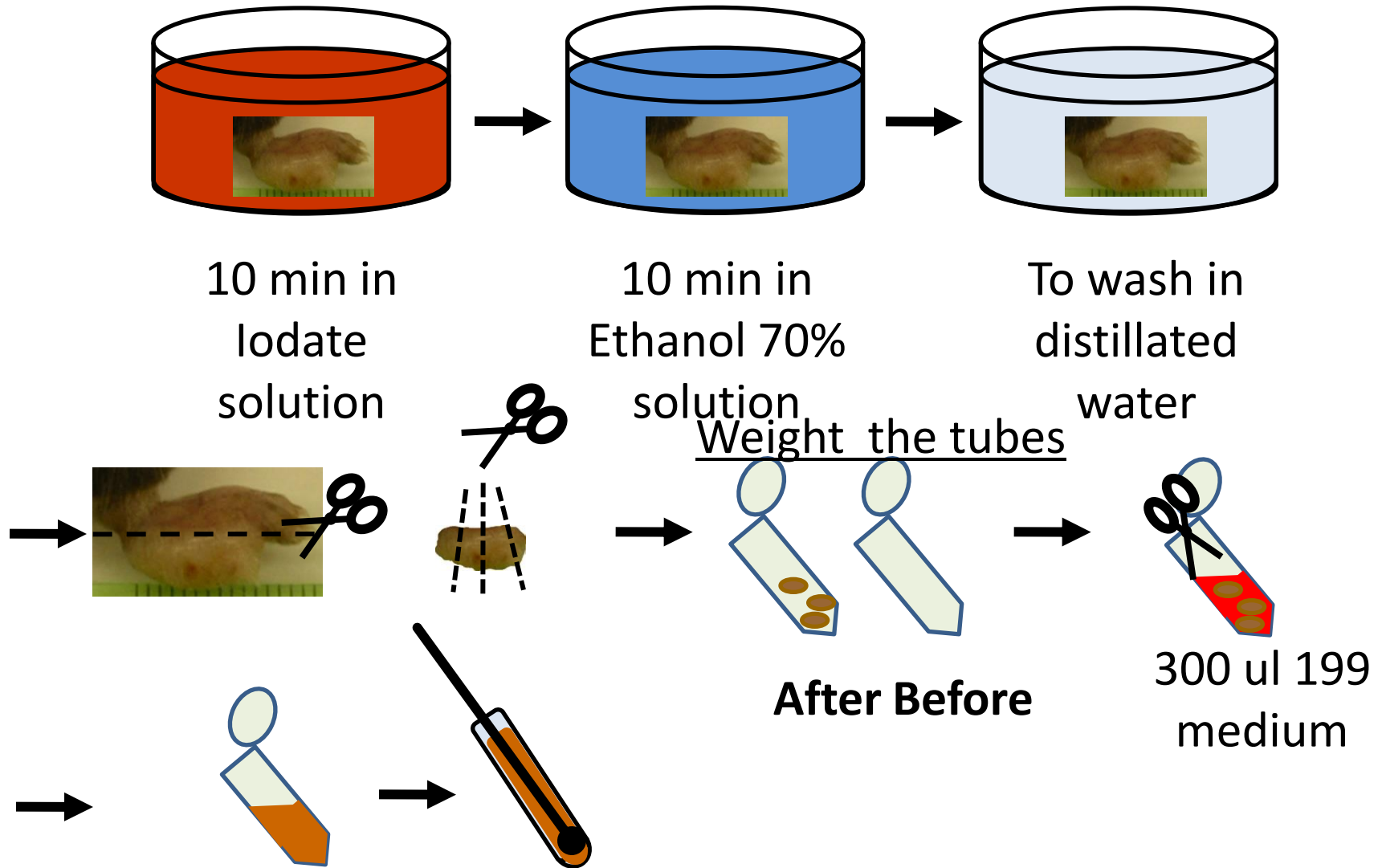
7 day: + 10  $\mu$ g CD200-Fc

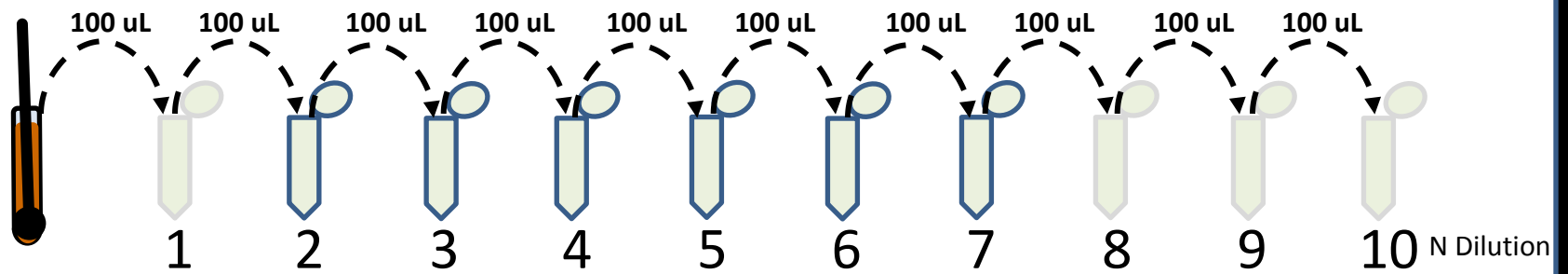
14 day: + 10  $\mu$ g CD200-Fc

21 day: + 10  $\mu$ g CD200-Fc

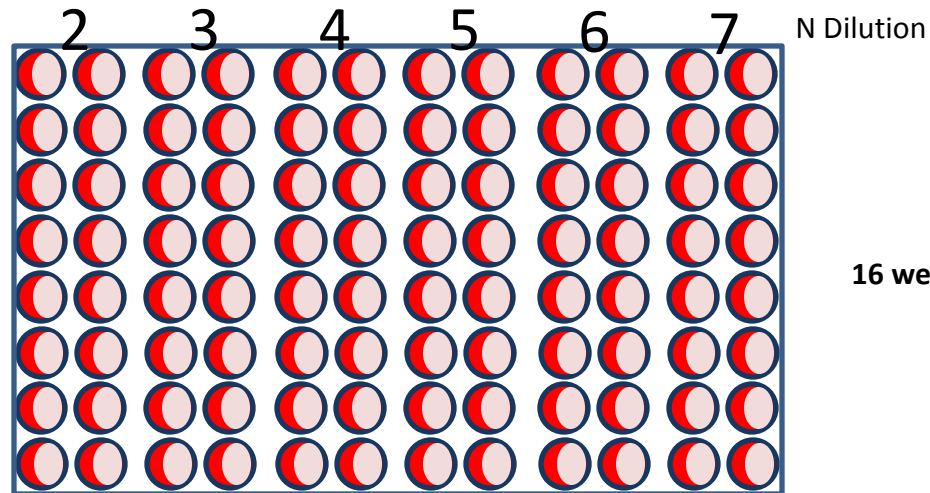
Etc...

# Quantification of parasite load in lesion: Protocol





- il. 2:  $n \text{ parasites} \times 100 \times 3$
- il. 3:  $n \text{ parasites} \times 10^3 \times 3$
- il. 4:  $n \text{ parasites} \times 10^4 \times 3$
- il. 5:  $n \text{ parasites} \times 10^5 \times 3$
- il. 6:  $n \text{ parasites} \times 10^6 \times 3$
- il. 7:  $n \text{ parasites} \times 10^7 \times 3$



16 wells/every dilution/50 ul

Third week

Example:

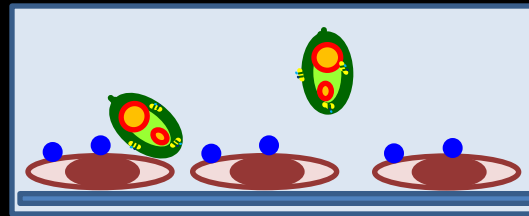
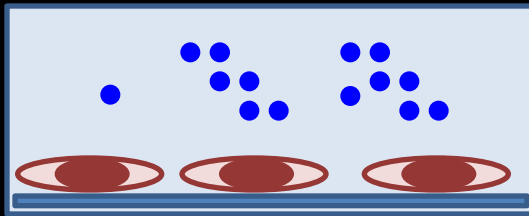
# Schematic procedure:

● CD200 molecule

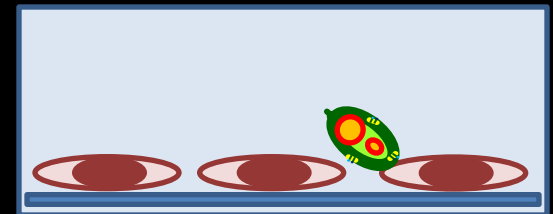
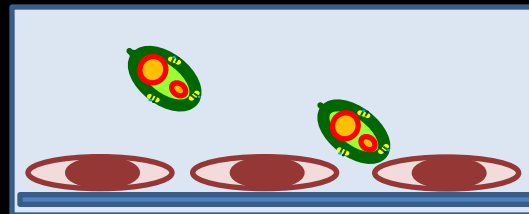
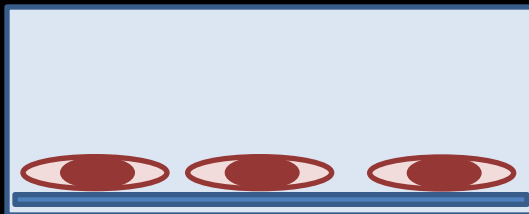
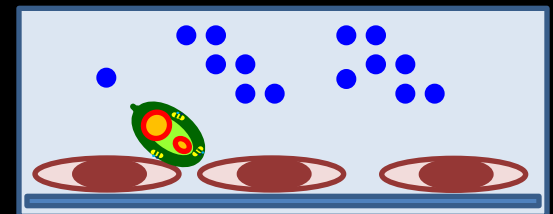
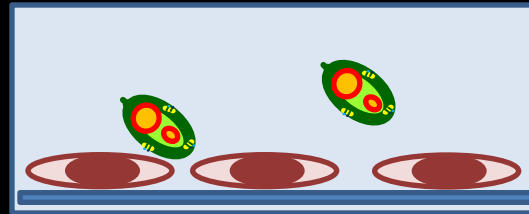
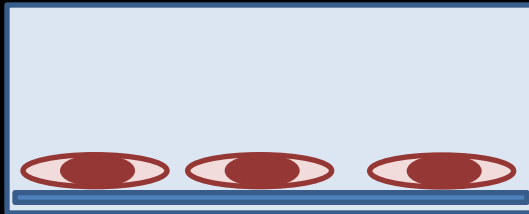
 *Leishmania*

 Macrophage

CD200: 4 $\mu$ g/mL



Wash out



# Schematic procedure:

Drugs:

- L-NMMN
- D-NMMN

