Regulatory T cells, Th17 cells and TLRs: crucial roles in the immunity to *Paracoccidioides brasiliensis* infection

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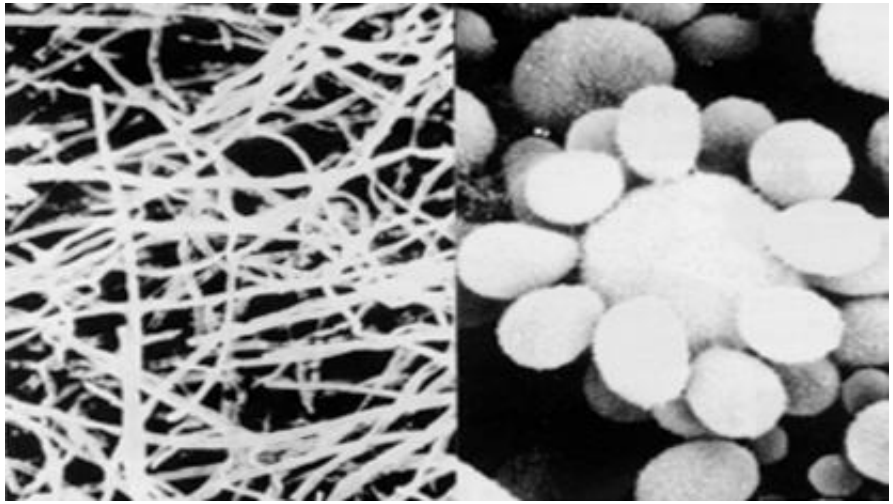
Paracoccidioides brasiliensis

(Thermally dimorphic fungus)

Mycelium

23-26°C



Yeast

35-37°C

- ✓ Pathogenicity appears to be linked to morphogenesis since strains unable to undergo the morphologic transition are not virulent

- ✓ Yeast cells are multinucleated (2-8 nuclei), assexual, unknown ploidy

- ✓ Very little is known about the biology of the fungus

  Pb genome recently sequenced and published (PLoS Genet, 2011)

PARACOCCIDIOIDOMYCOSIS (Adolpho Lutz, 1908)

Paracoccidioides brasiliensis

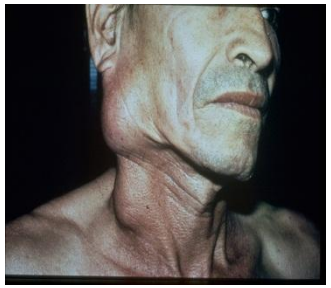
Infection route: inhalation of air-borne spores

PCM-infection

asymptomatic pulmonary infection
(10 million people infected in LA)
(Th1 pole of Immunity)

PCM Disease:

wide spectrum of clinical manifestations
Severe (Th2/Th3), Benign (Prevalent Th1)



Lymphatic
Disseminated Form

Cutaneous Lesion

Cutaneous
Disseminated Lesions

Endemic Region:
Latin America

Isogenic Murine Model of PCM

(Mimics the Polar Forms of the Disease)

A/J:

RESISTANT

Positive DTH Responses

MΦActivation

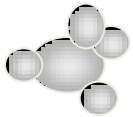
Organized Lesions

REGRESSIVE DISEASE

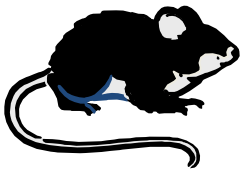
No Differences in Mortality

Late Th1

- **IL-2**
- **IFN-γ**



P b



B10.A

SUSCEPTIBLE

T Cell Anergy

No MΦ Activation

Non-Organized Lesions

PROGRESSIVE DISEASE

Increased Mortality Rates

Th2?

- **No IL-4**
- **IL-5**
- **Early IL-10**
- **No IFN-γ**
- **No IL-2**

Calich et al, Infect Immun. Brit. J. Exp. Pathol.,66, 584, 1985

Cano &Calich, . Infec.& Immun., 66:800 - 806, 1998.

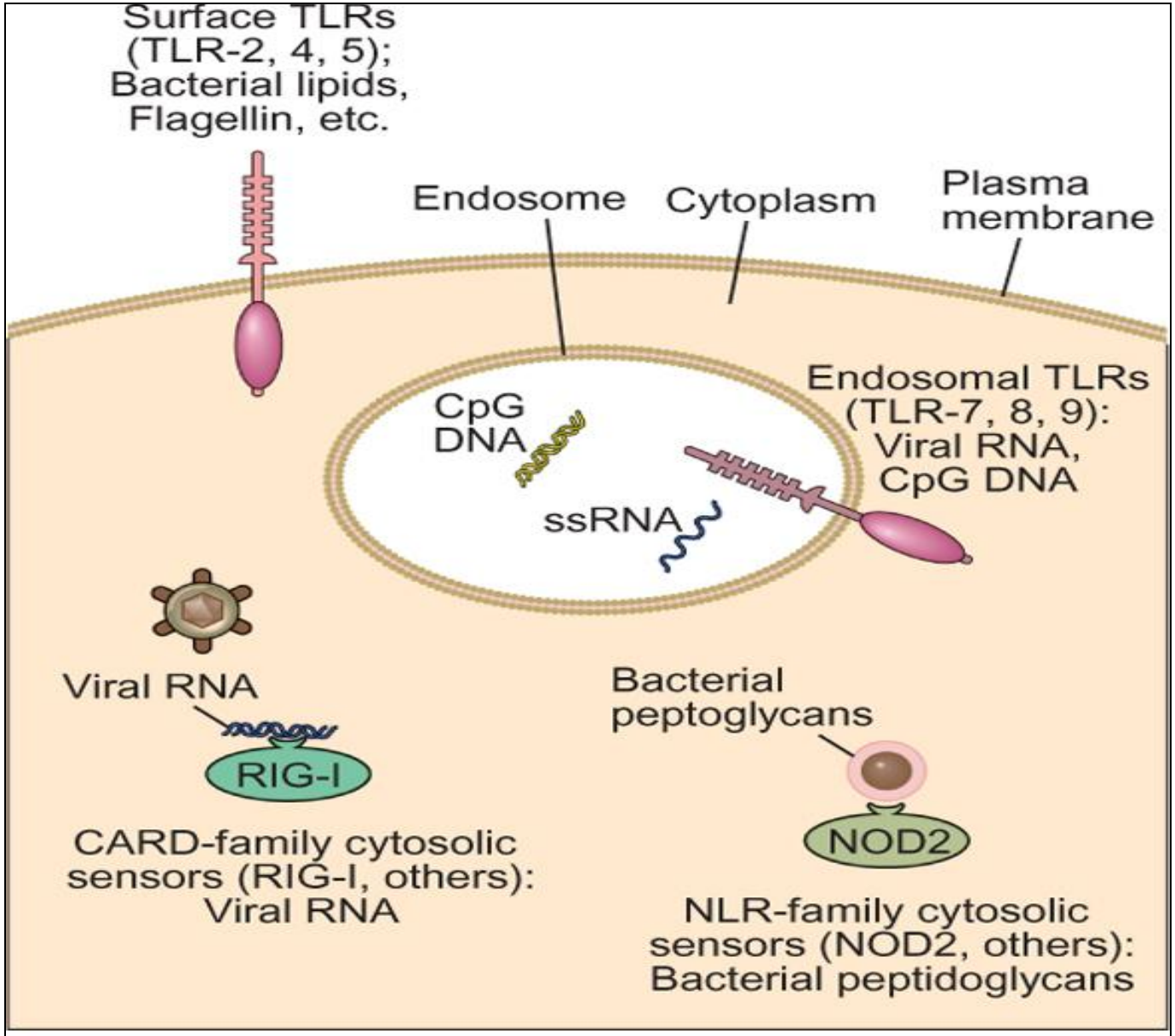
Why studies on Innate immunity against *P. brasiliensis*?

- **Recent studies : mechanisms of Innate Immunity (initial immune response of hosts) shape the adaptive immune response and determines disease outcome**

- **Human paracoccidioidomycosis is diagnosed late in infection, thus innate immunity is totally unknown**

- **Experimental models are instrumental to characterize Innate Immunity and determine its influence on adaptive immunity and severity of disease**

Pattern Recognition molecules of the innate immune system



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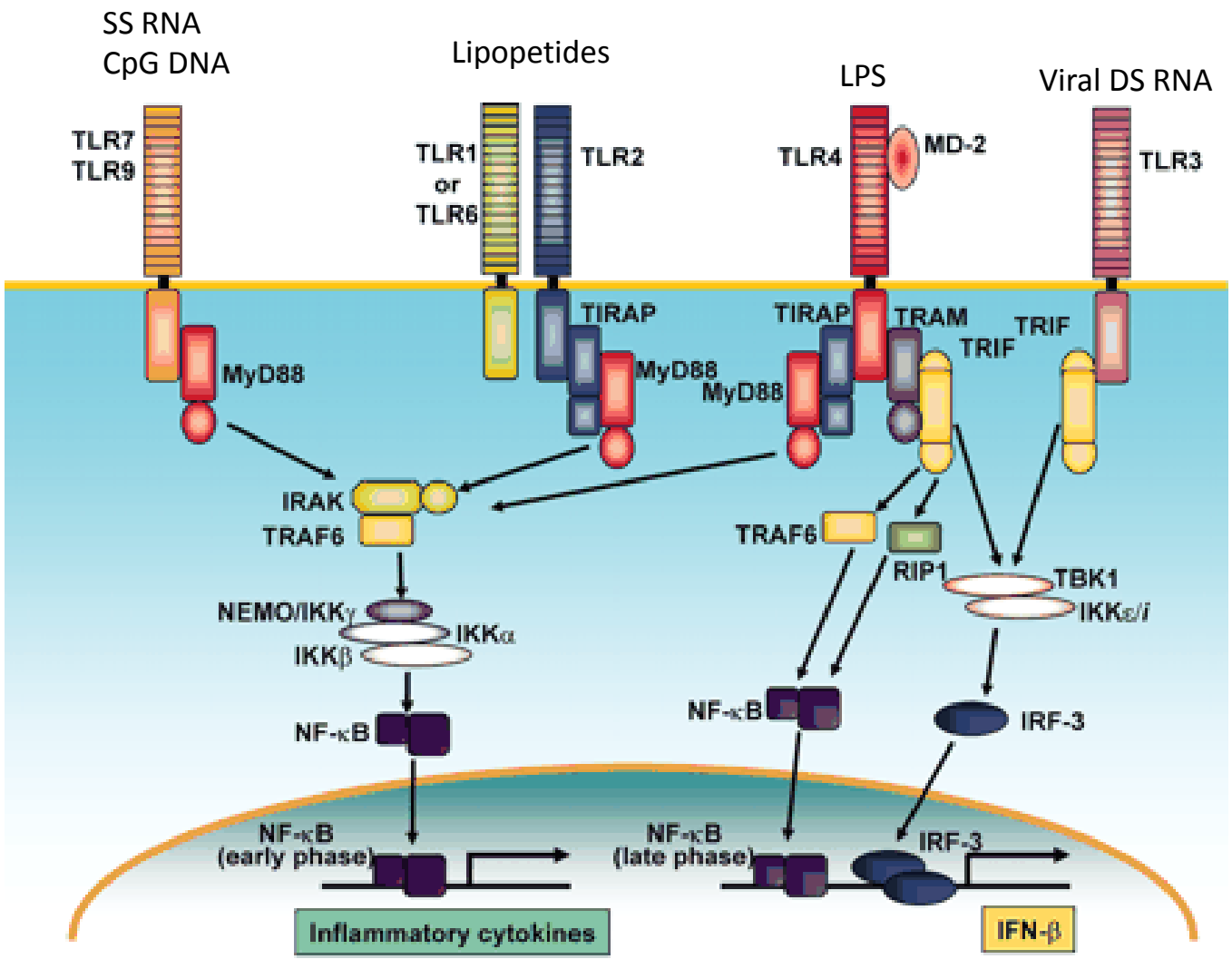
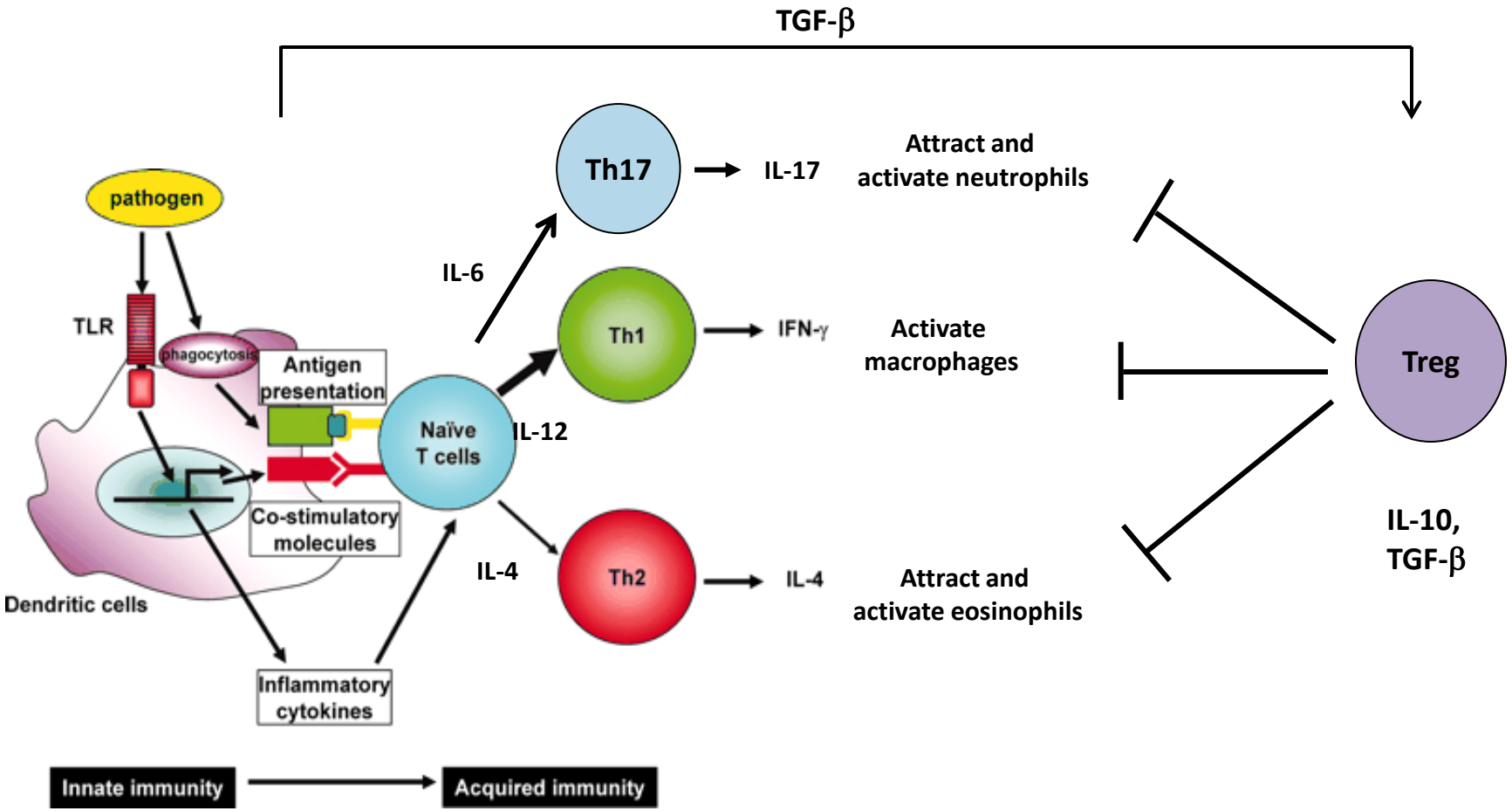
TLR- Signaling Pathways



Takeda & Akira, Intern. Immun.,17: 1, 2005

TLRs and DCs Exert Major Influence in Innate and Adaptive Immunity



Modified from Takeda & Akira, Intern. Immun.,17: 1, 2005

ROLE of TLR2, TLR4 and MyD88 in PULMONARY PCM
(Studies In Vitro and In Vivo)

Mouse Strains WT X TLR2KO, TLR4KO, MyD88KO, Dectin KO Mice*

In vitro Studies

Normal Mϕ
or DCs (WT)

Deficient Mϕ
or DCs

+ Pb (Co-cultivation)

↓

Phagocytosis
Killing
NO + Cytokines
Costimulatory Molecules
APC Ability

In vivo Studies

WT X TLR2KO, TLR4KO, MyD88KO, Dectin KO

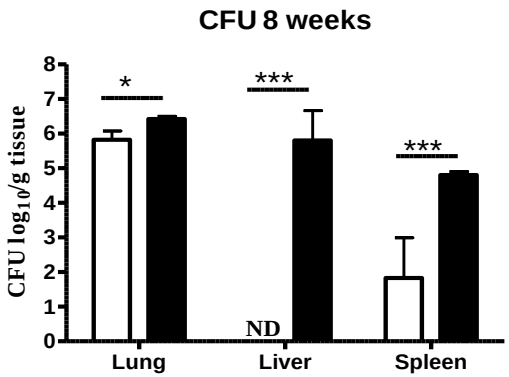
1x 10^6 Pb (i.t.)

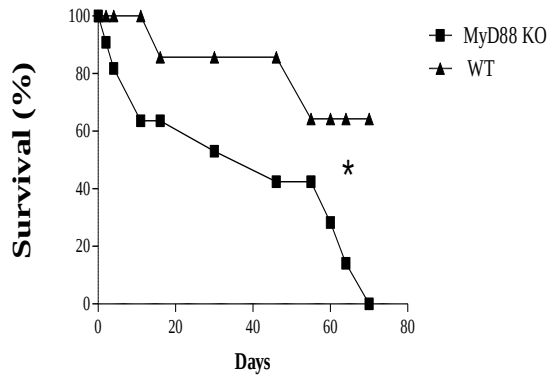
↓ 72-96h
11-Weeks

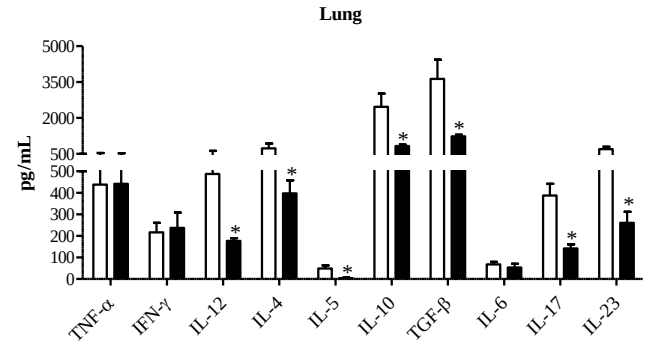
CFU, Cytokines, NO,
Lung Leukocytes Phenotyping
Lymphoproliferation
Histopathology

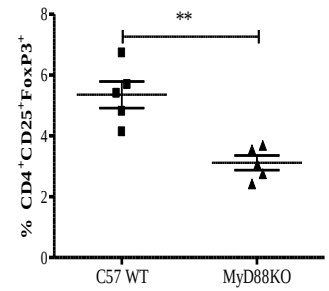
* kindly provided by Prof. Akira and G. Brown

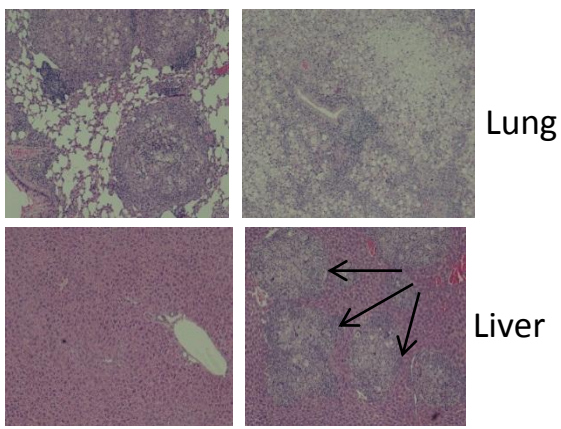
MyD88 Deficiency Results in Severe infection, and High Mortality









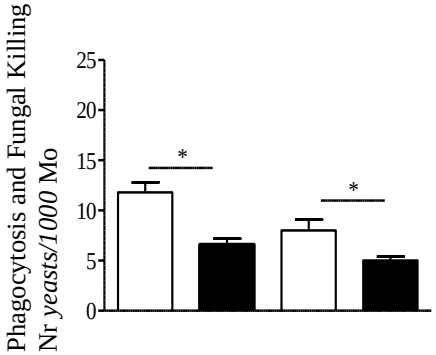


Absence of MyD88 signaling results in:

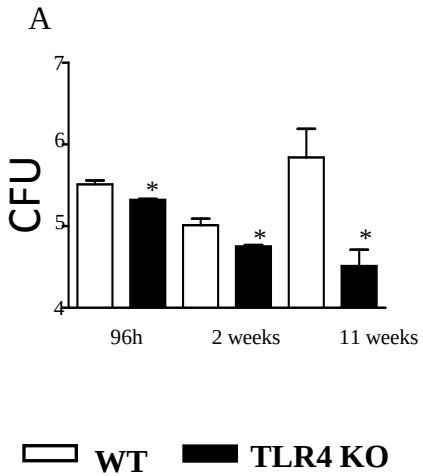
- More severe in infection and organ pathology
- Decreased Th1/Th2/Th17 immunity
- Increased mortality rates
- Decreased influx of T cells to the site of infection
- Decreased expansion of Treg cells

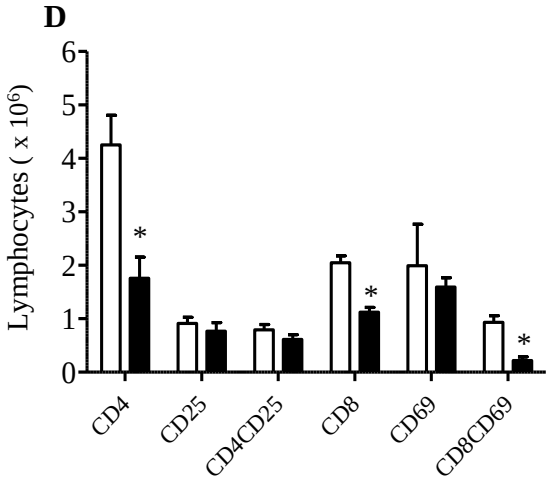
Loures F. et al. Infect & Immun. 79, 2470-2480, 2011

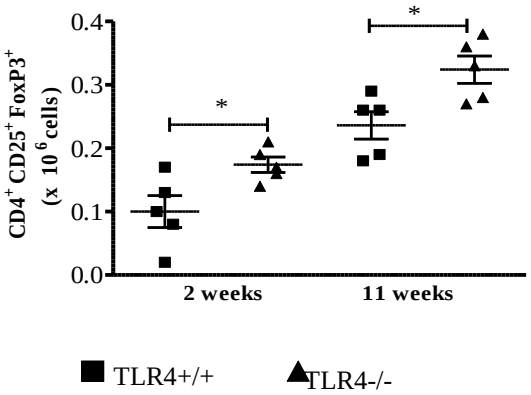
Impaired TLR4 signaling induces decreased fungal loads, reduced Th17 immunity but increased Treg cells

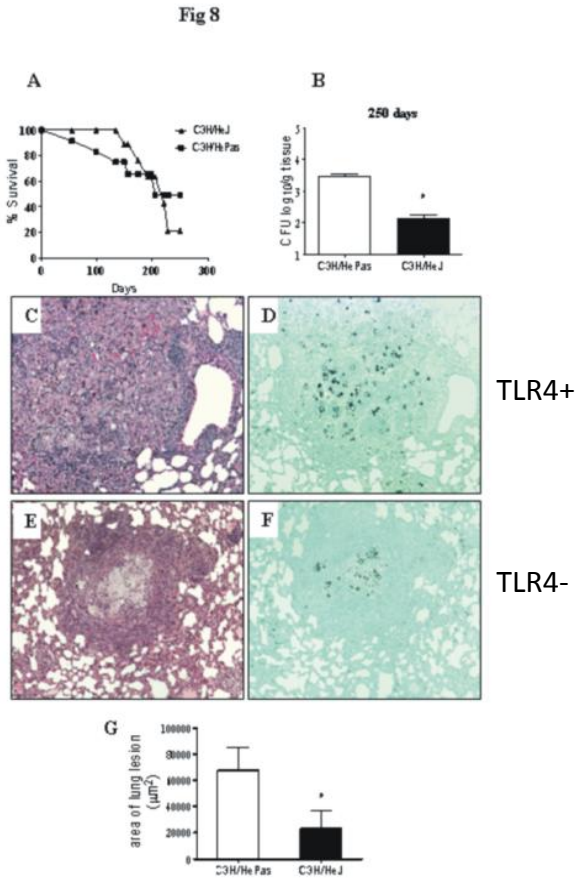


Low IL-12, IFN-γ, IL-6 and IL-23
Impaired Th1/Th17



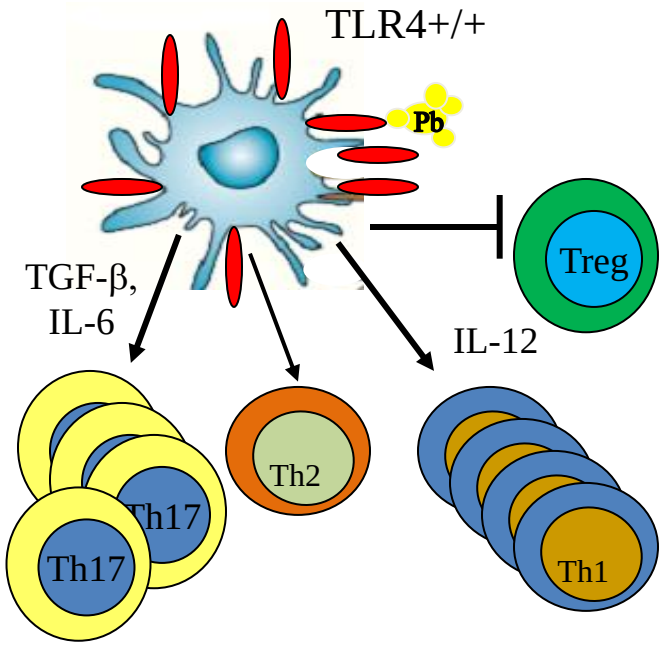




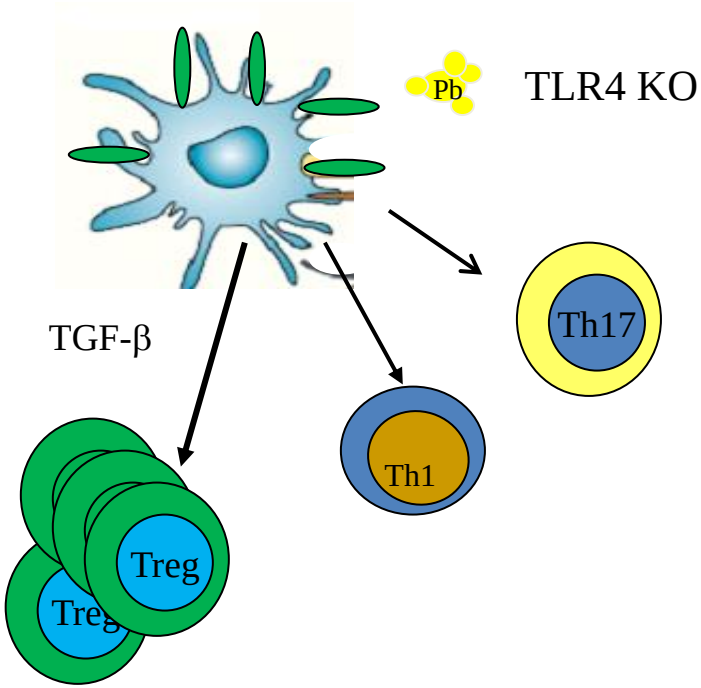


Loures et al., Infect & Immun. 78: 1078-1088, 2010.

CONCLUSION: TLR4 Is a Positive Regulator of Th17 Cells and Tissue Inflammation in Pulmonary Paracoccidioidomycosis



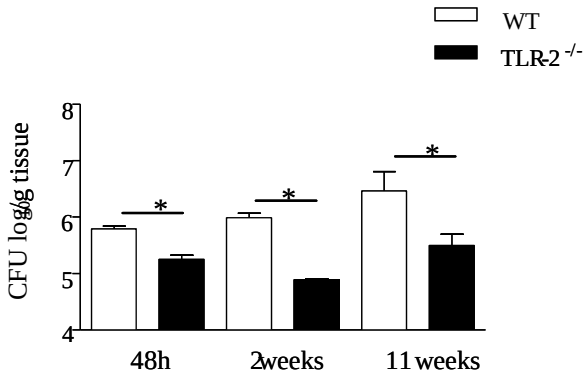
Increased Th17/Th1
Immunity and Inflammation

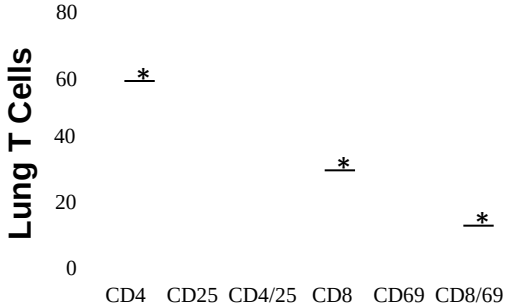


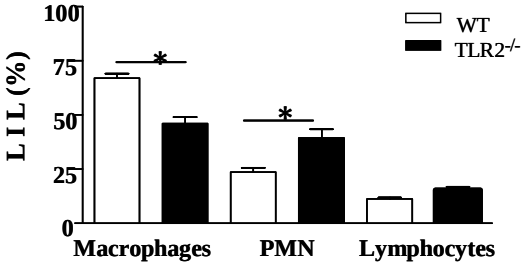
Reduced Th17/Th1 Immunity, High Tregs
Low Inflammation
Decreased Tissue Damage

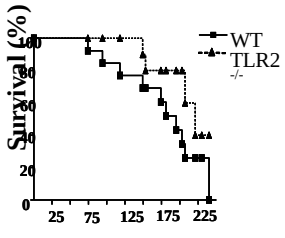
Loures FV, Pina A, Felonato M, Calich VLG. Infect. Immun. 78: 1078-1088, 2010

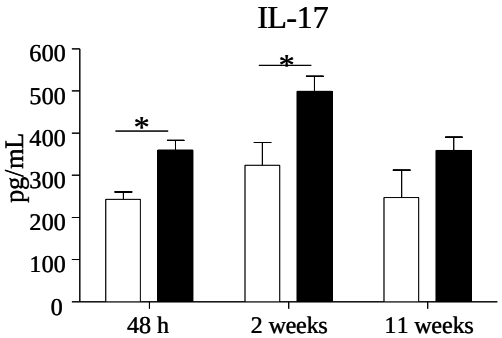
TLR2 $^{-/-}$ Mice Have Less Severe Infection Controlled by PMNs and Th17 Cells, but Increased Inflammation and Lung Pathology

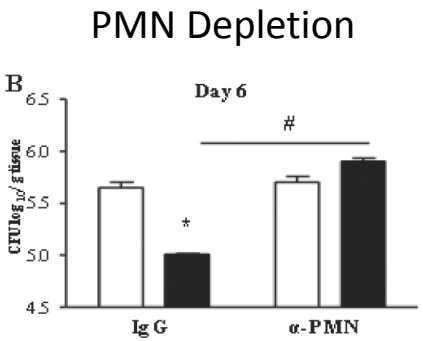


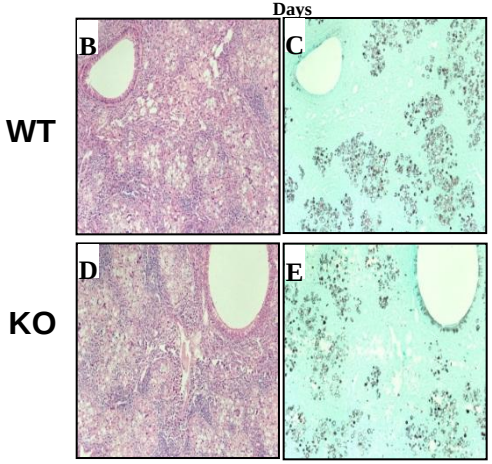


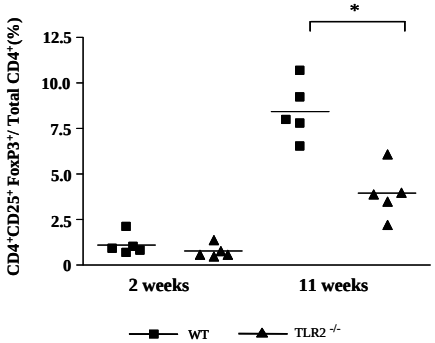


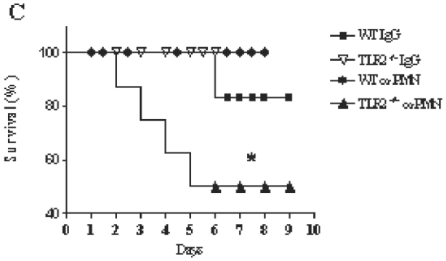






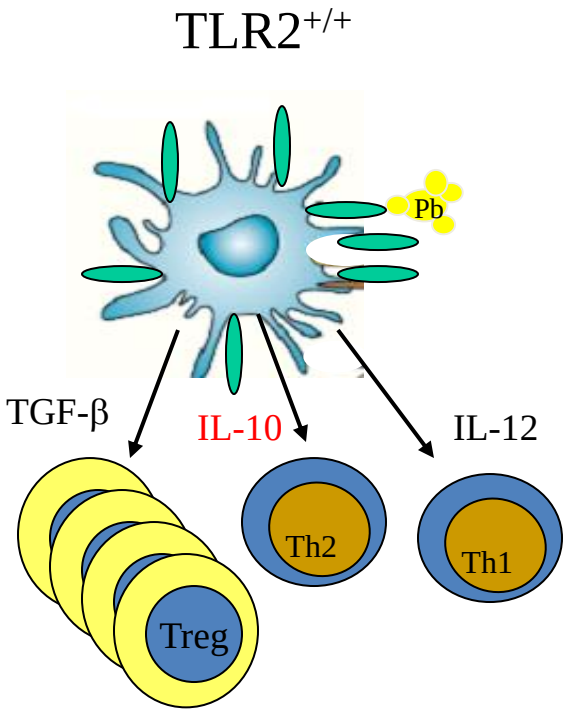




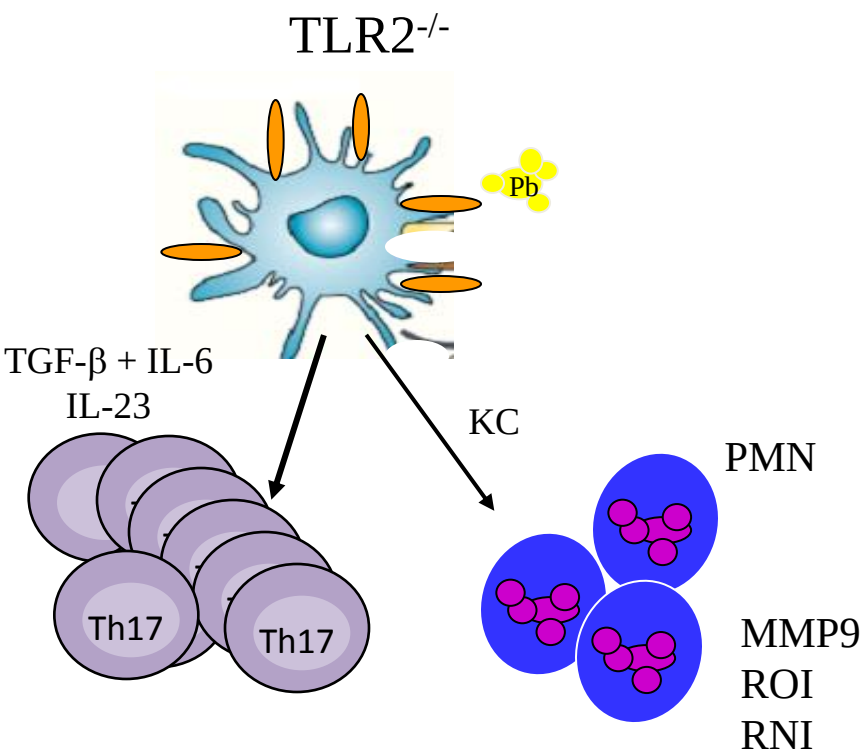


Loures FV, Pina A, Felonato M, Calich VLG. J. Immunol. 183, 1279-90, 2009.

CONCLUSION: TLR2 Is a Negative Regulator of Th17 Cells and Tissue Pathology in Pulmonary Paracoccidioidomycosis



Treg-controlled
T cell immunity

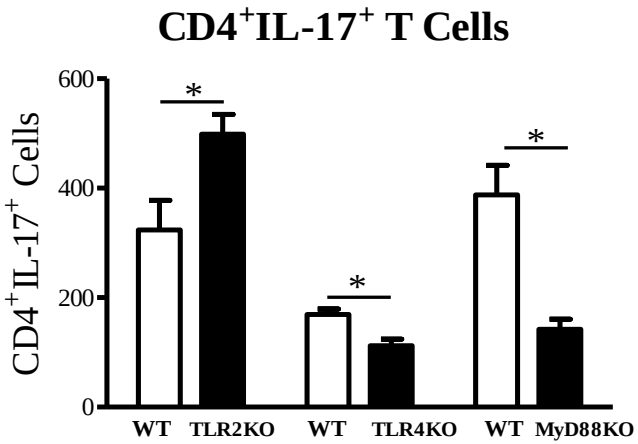


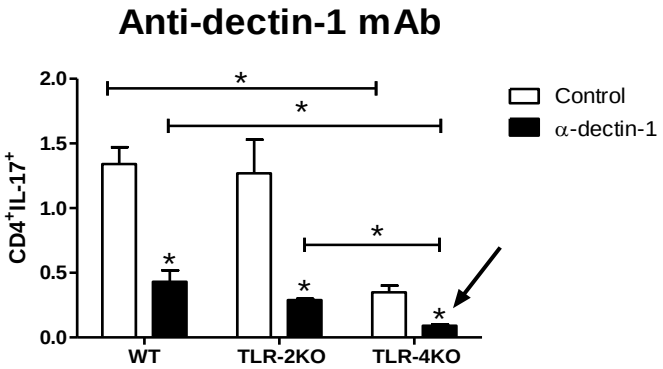
Th17 Immunity
High Inflammation
Tissue Damage

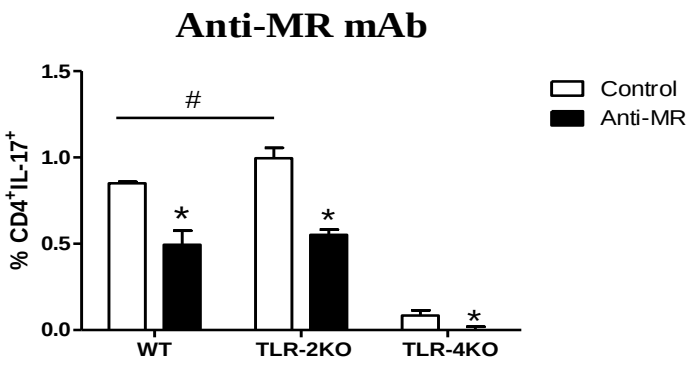
Loures FV, Pina A, Felonato M, Calich VLG. J. Immunol. 183, 1279-90, 2009.

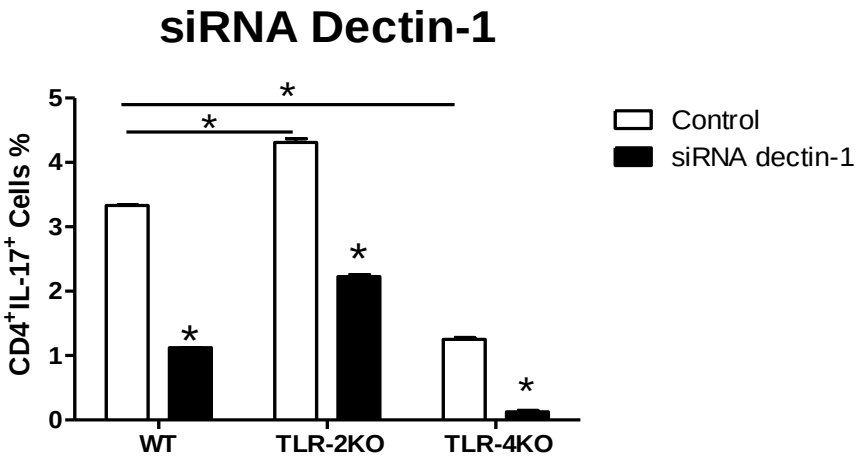
Mannose Receptors (MR) and Dectin-1 Signaling Control Th17 Expansion by DCs

DCs + Pb + Naïve Lymphocytes ⟶ $CD4^+IL\text{-}17^+$ Cells by Facs

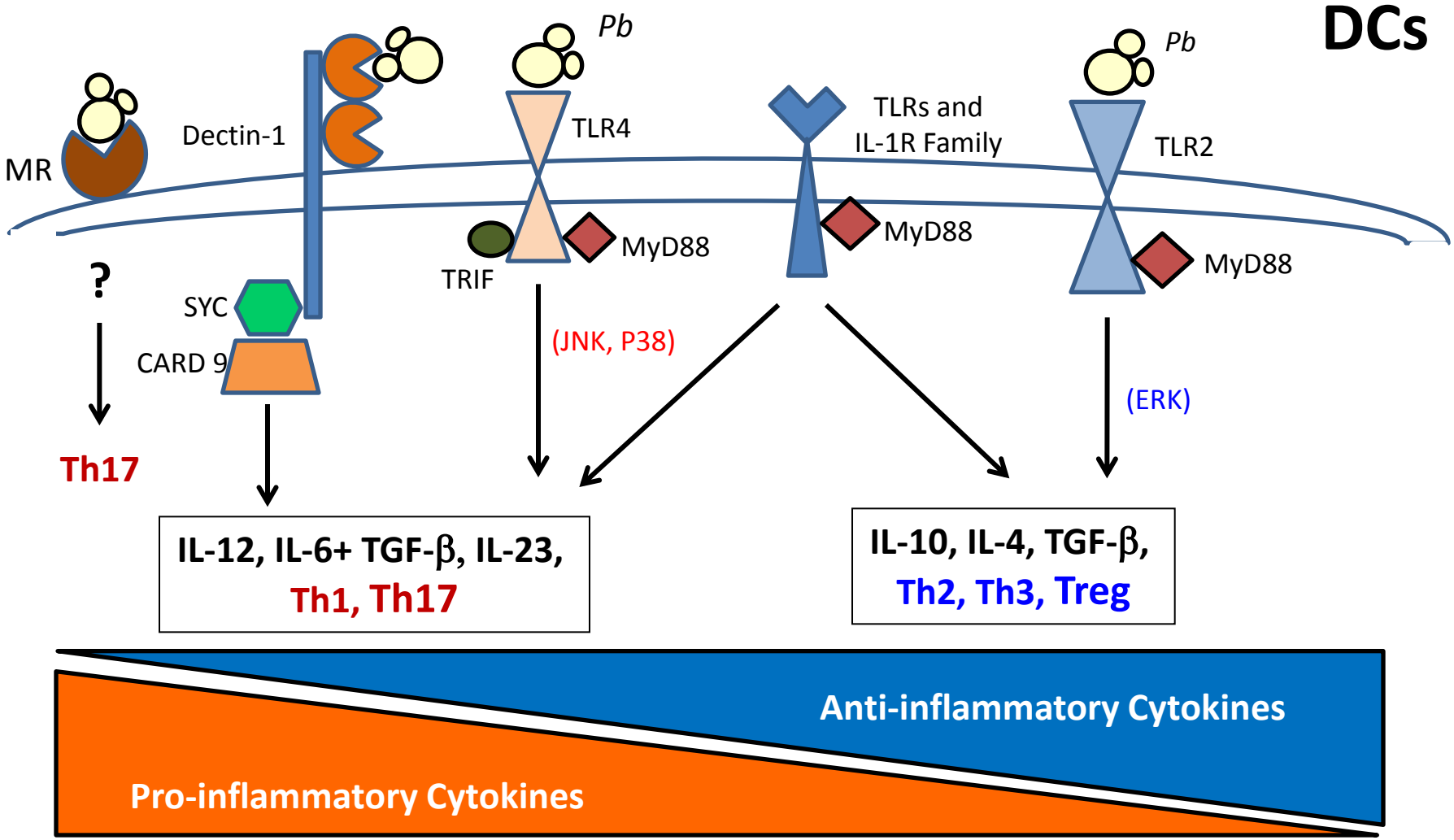








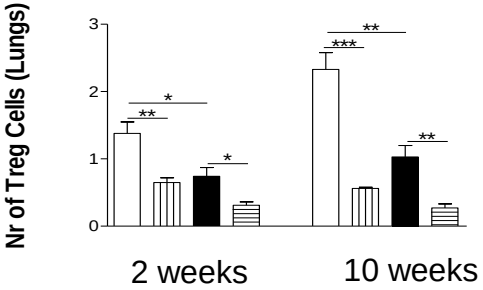
P. brasiliensis Induces Pro- and Anti-Inflammatory Responses Using TLRs and CLRs



Calich et al. FEMS Immunol, 2008;
Calich et al. Mycopathol, 2008

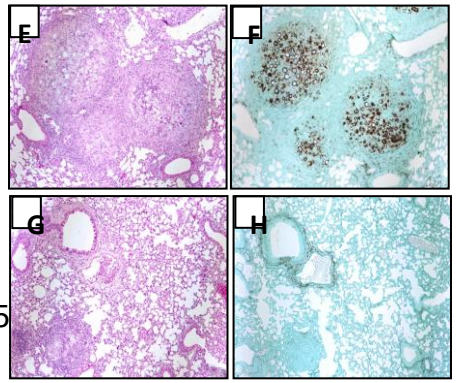
Depletion of Treg Cells Improves the Disease of Resistant and Susceptible Mice

Anti-CD-25 depletes Treg cells

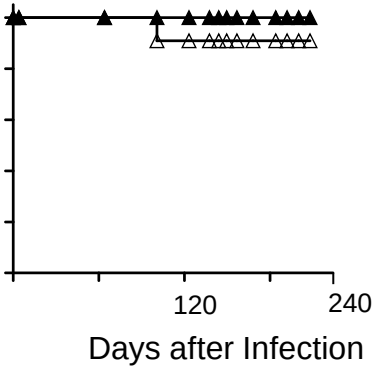


B10.A Ig

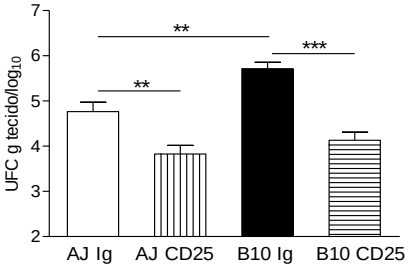
B10.A α-CD25



A/J

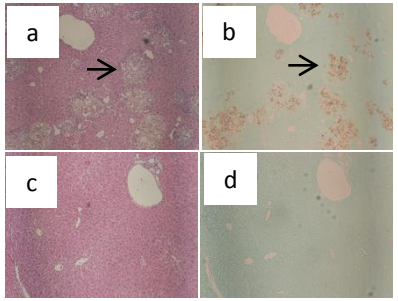


Fungal Loads, Lung

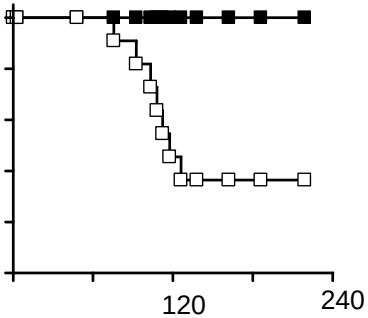


B10.A Ig

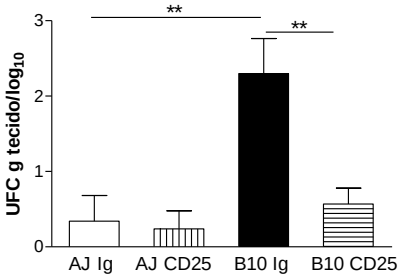
B10.A α-CD25



B10.A



Fungal Loads, Liver

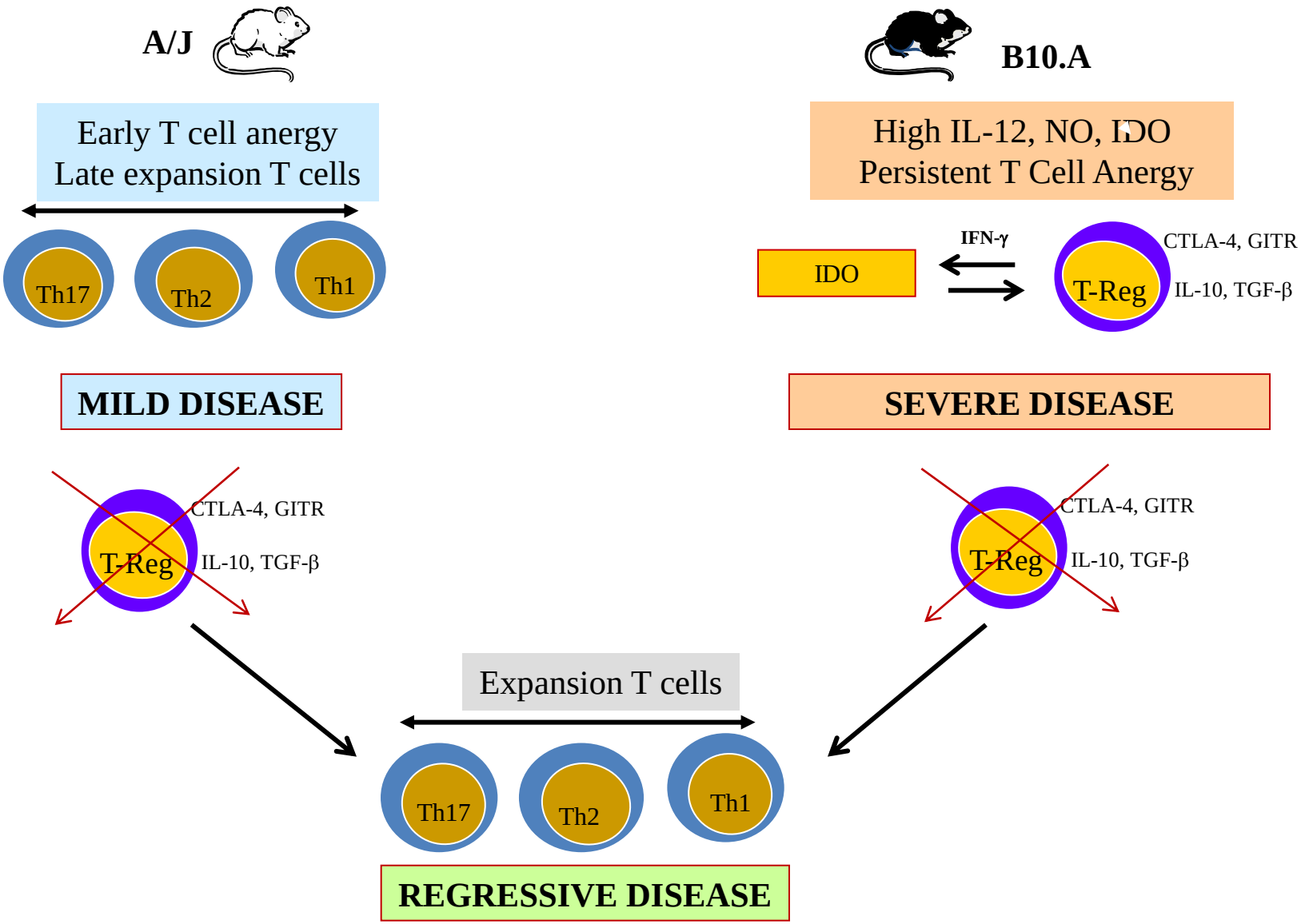


Early Increased Influx of T Cells, B Cells, Mϕ

High Levels Th1/Th2/Th17 Cytokines

Felonato & Calich, submitted, 2011

Depletion of Treg Cells Improves the Mild and Severe Forms of PCM



CONCLUSIONS

- Mechanisms of Innate Immunity define the adaptive immunity against Pb
- MyD88 adaptor molecule define disease outcome by regulating the production of pro- and anti-inflammatory cytokines and expansion of all T cell subsets during Pb infection
- TLR4 and TLR2 exert opposing roles in PCM: TLR4 signaling induces pro-inflammatory responses whereas TLR2 induces anti-inflammatory responses
- CLRs (MR and Dectin-1) signaling induce Th17-biased immunity
- Treg cells exert deleterious effects in the mild and severe forms of PCM

Characterize the molecular and cellular basis of hosts responses against this endemic fungal infection will bolster efforts to develop effective vaccines, rapid diagnostics, and new kinds of therapeutics.

Acknowledgements

Prof. Shizuo Akira and Prof. Gordon Brown for kindly providing TLR, MyD88 and Dectin deficient mice

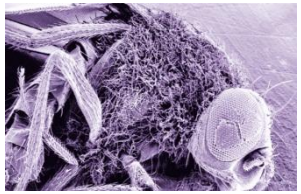
- Flávio V. Loures: PCM Studies in TLR and MyD88-Deficient Mice

- Adriana Pina, Simone Bernardino, Maíra Felonato, Alexandre Keller for the collaboration in several studies

- Fapesp : Financial Support

Nobel Prize in Medicine 2011

Jules Hoffman



Described the role of Toll in anti-fungal defense

Bruce A. Beutler

Identified TLR4 as LPS Receptor

Ralph Steinman

Discovered the dendritic cells that play a critical role in activating the body's adaptive immune system,