New advances in the development of a vaccine against *Paracoccidioides* spp.

Carlos Pelleschi Taborda

Institute of Biomedical Sciences – Department of Microbiology
Medical Mycology Laboratory-IMTSP/LIM53
University of São Paulo

Why we should study different tools for treatment of systemic mycoses?

- 1,7 bilhões de pessoas – Infecções fúngicas
- 1-2 milhões de pessoas - Infecções fúngicas invasivas
- Altas taxas de mortalidade

Table 1. Statistics of the 10 most significant invasive fungal infections.

Disease (most common species)	Location	Estimated life-threatening infections/ year at that location*	Mortality rates (% in infected populations)*
Opportunistic invasive mycoses			
Aspergillosis (*Aspergillus fumigatus*)	Worldwide	>200,000	30–95
Candidiasis (*Candida albicans*)	Worldwide	>400,000	46–75
Cryptococcosis (*Cryptococcus neoformans*)	Worldwide	>1,000,000	20–70
Mucormycosis (*Rhizopus oryzae*)	Worldwide	>10,000	30–90
Pneumocystis (*Pneumocystis jirovecii*)	Worldwide	>400,000	20–80
Endemic dimorphic mycoses*†			
Blastomycosis (*Blastomyces dermatitidis*)	Midwestern and Atlantic United States	~3,000	<2–68
Coccidioidomycosis (*Coccidioides immitis*)	Southwestern United States	~25,000	<1–70
Histoplasmosis (*Histoplasma capsulatum*)	Midwestern United States	~25,000	28–50
Paracoccidioidomycosis (*Paracoccidioides brasiliensis*)	Brazil	~4,000	5–27
Penicilliosis (*Penicillium marneffei*)	Southeast Asia	>8,000	2–75

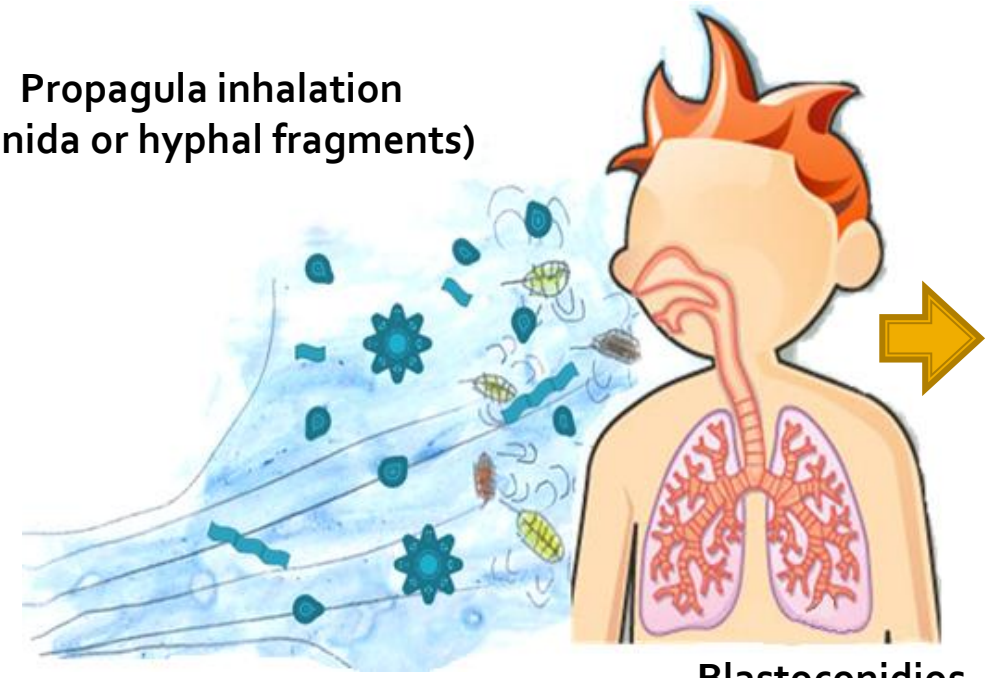
*Most of these figures are estimates based on available data, and the logic behind these estimates can be found in the text and in the Supplementary Materials. †Endemic dimorphic mycoses can occur at many locations throughout the world. However, data for most of those locations are severely limited. For these mycoses, we have estimated the infections per year and the mortality at a specific location, where the most data are available.

Brown et al. Sci Transl Med 4, 165rv13, 2012.

The infective particles "conidia" are produced by the mold, which one to be inhaled reach the alveoli of the susceptibel host, There its have the capacity to transform into the yeast (pathogenic phase).

Transformation capacity in those fungus made them pathogenic agents able to produce deep mycoses.

Propagula inhalation (conida or hyphal fragments)



Aerosol generation

Blastoconidios Transformation

Balance Host - Pathogen

- **Host**
  - **Immunological status**
  - **Lung integrity**
- **Fungi**
  - **Inoculum**
  - **Fungus virulence**

Kauffman CA, et al. Chest Med, 2009 / Deepe G, et al, Med Mycol, 2005

Treatment for dimorphic fungal pathogens

Antifungal drugs

- -Polyenes
- -Imidazoles
- -Triazoles
- -Echinocandins

→ sulphamethoxazole/ trimethoprim

Essential oils/ Natural products

Vaccine/Immunotherapy???

Treatment for dimorphic fungal pathogens

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REVIEW

Antifungal therapeutics for dimorphic fungal pathogens

Kristie D. Goughenour and Chad A. Rappleye

Department of Microbiology, Ohio State University, Columbus, OH, USA

Table 1. In vitro antifungal MICs for dimorphic fungal pathogens.

		MIC range (μg/mL)			
Drug class	Antifungal	*Histoplasma*	*Blastomyces*	*Paracoccidioides*	*Coccidioides*
Polyenes	Amphotericin B	Y: <0.03–2.0 M: 0.26–2.5	Y:<0.03–2.0 M:	Y: 0.06–2.0 M:	Y: 0.25–2.0 M: 0.03–0.50
Imidazoles	Ketoconazole	Y: M: 0.17	Y: <0.01–0.25 M: 0.1–0.4	Y: <0.01–0.03 M:	Y: M: 0.03–0.16
Triazoles	Fluconazole	Y: 0.25–8.0 M: 2.0–32	Y: 0.06–32 M: 0.06–32	Y: 0.13–0.50 M:	Y: M: 2.0–64
	Itraconazole	Y: <0.01–0.5 M: 0.03–1.0	Y:<0.01–0.13 M: 0.03–4	Y: <0.01–0.06 M:	Y: <0.03–0.50 M: 0.03–1.0
	Voriconazole	Y: 0.03–0.50 M: <0.01–2.0	Y:<0.03–0.25 M: 0.06–2.0	Y: M:	Y: <0.03–2.0 M: 0.03–1.0
	Posaconazole	Y: <0.01–0.50 M: 0.02–2.0	Y: <0.02–0.06 M: <0.02–2.0	Y: M:	Y: M: 0.06–1.0
Echinocandins	Micafungin	Y: >64 M: 0.03–0.06	Y: 32–64 M: <0.01–0.03	Y: >64 M: 4–16	Y: M: 0.02
	Caspofungin	Y: 8–32[a] M: 0.02–4.0	Y: M: 0.5–8.0	Y: M:	Y: M: 8–64

Why develope a vaccine against *Paracoccidioides* spp.?

Mem Inst Oswaldo Cruz, Rio de Janeiro, Vol. *104*(3): 513-521, May 2009 513

Mortality due to systemic mycoses as a primary cause of death or in association with AIDS in Brazil: a review from 1996 to 2006

Marli Prado[1], Marcelo Barbosa da Silva[2], Ruy Laurenti[3], Luiz R Travassos[3], Carlos P Taborda[2]/+

[1]Instituto de Ciências Biomédicas, Departamento de Microbiologia e Laboratório de Micologia Médica - LIM53 - Hospital de Clínicas da Faculdade de Medicina [2]Departamento de Epidemiologia, Escola de Saúde Pública, Universidade de São Paulo, São Paulo, SP, Brasil [3]Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, São Paulo, SP, Brasil

TABLE I

Number and frequency of deaths per systemic mycosis (3C - ICD - 10). Brazil, annual average, 1996-2006[a]

	Annual average 1996-1998		Annual average 1999-2001		Annual average 2002-2004		Annual average 2005-2006[a]	
Systemic mycosis (ICD-10)	n	%	n	%	n	%	n	%
Blastomycosis + Paracoccidioidomycosis	171	53.9	173	55.3	175	50.9	148	44.6
Cryptococcosis	78	24.5	76	24.3	81	23.5	89	26.8
Candidiasis	39	12.2	36	11.5	51	14.9	54	16.3
Histoplasmosis	15	4.8	10	3.2	12	3.6	19	5.6
Aspergillosis	12	3.8	13	4.1	18	5.3	17	5.0
Zygomycosis	2	0.5	4	1.3	4	1.3	3	0.9
Coccidioidomycosis	1	0.3	1	0.3	2	0.6	3	0.8
Total	317	100.0	312	100.0	345	100.0	331	100.0

a: preliminary data for 2006.

TABLE VIII

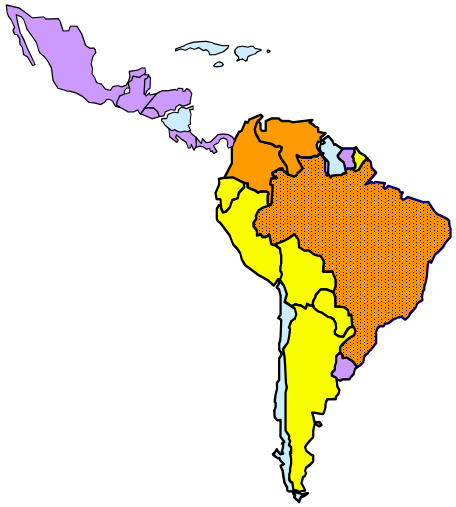
Number and frequency of mentions to systemic mycoses in deaths with underlying cause of AIDS in Brazil from 1996-2006[a]

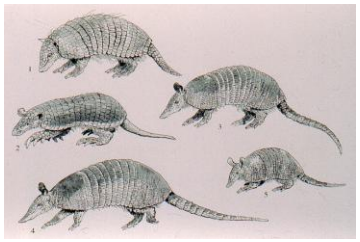
Mention (3C - ICD - 10)	n	%	Number per 1,000 deaths of AIDS
B45 Cryptococcosis	3001	50.9	23.89
B37 Candidiasis	1780	30.2	4.17
B39 Histoplasmosis	594	10.1	4.73
B44 Aspergillosis	427	7.2	3.40
B40 e B41 (Blastomycosis/Paracoccidioidomycosis)	84	1.4	0.67
B38 Coccidioidomycosis	11	0.2	0.09
B46 Zygomicosis	1	0.0	0.01
Total (systemic mycoses)	5.898	100.0	46.95

a: preliminary data for 2006. Obits by AIDS as basic cause of death (Cod B20-24 - Chapter I - same infectious and parasitic disease - ICD - 10). Mention to systemic mycosis anywhere in part I or II of obit declaration with AIDS as the basic cause of death.

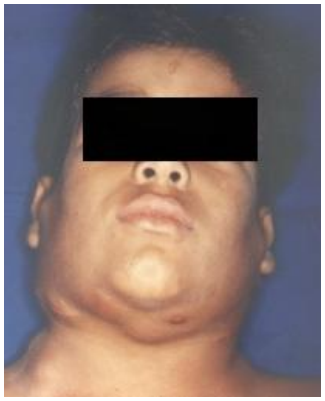
Paracoccidioidomycosis

Paracoccidioides spp. (*P. brasiliensis* and *P. lutzii*)

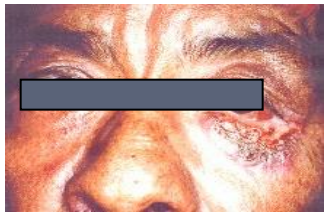


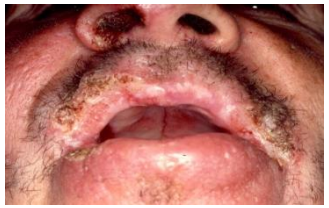


Mycelium
Conidia



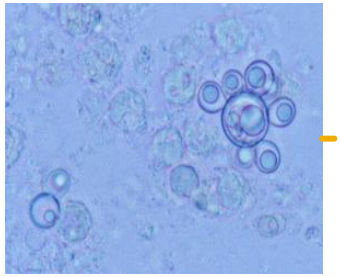
Acute PCM



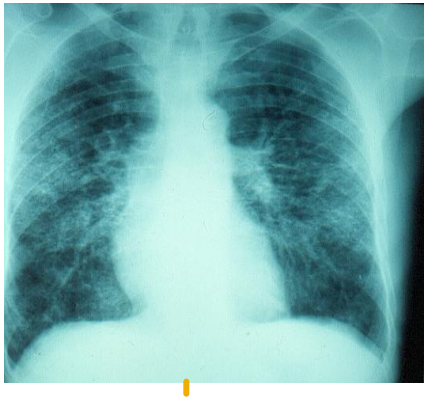


Chronic PCM

Dissemination: Mucous membranes, skin, lymphoid nodules, adrenal glands, and other organs.



Yeast



Treatment

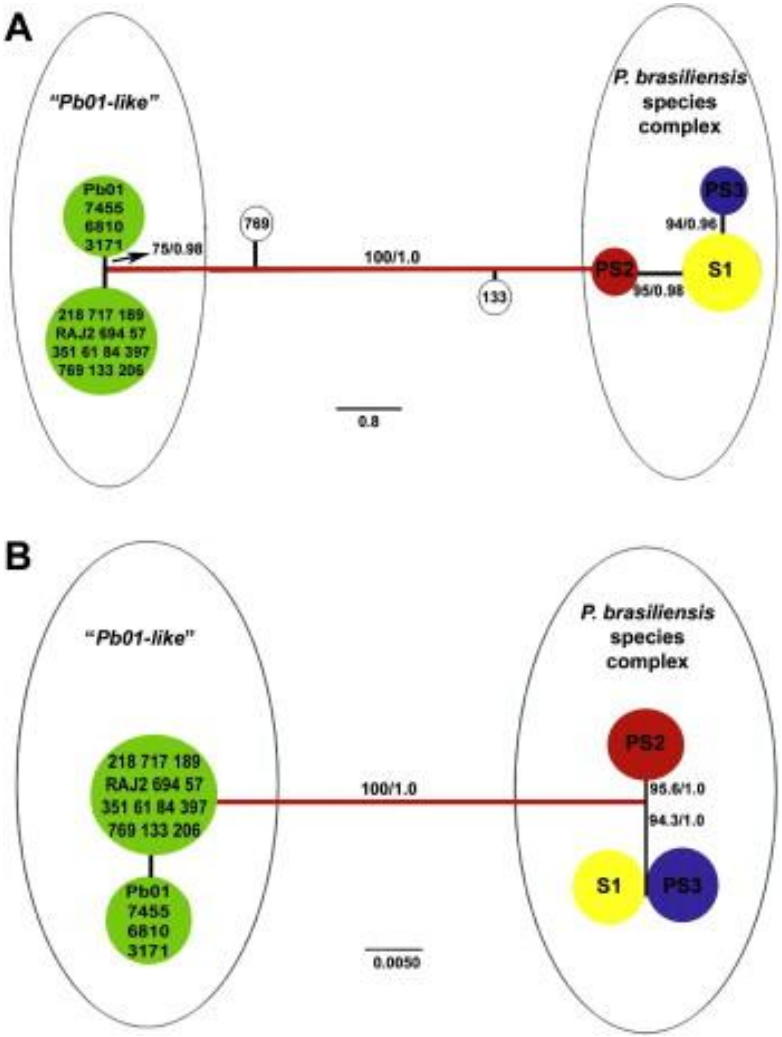
Itraconazole

Amphotericin B

sulfametoxazol-trimetoprim

Residual fibrotic lesions are observed in ~60%, of patients with PCM.

Phylogenetic Analysis



Vaccine against fungal infection

Table 1

A list of fungal vaccine candidates.

Fungus	Candidate	Subunit/whole	Immunity	Model	Reference
Aspergillus	Asp 16 f	Recombinant/subunit	Th1	Murine	[45]
Aspergillus	Asp 3 f	Recombinant/subunit	Th1	Murine	[46]
Aspergillus	Pep1p, Gel1p, Crf1, glucans	Recombinant/subunit	Th1	Murine	[47]
Aspergillus, Candida	Cell wall glucanase, Crf1	Recombinant	Th1	Murine	[12••]
Aspergillus, Coccidiodes, Cryptococcus, Candida	Heat-killed *Saccharomyces cerevisiae* (HKY)	Heat killed-Whole	(Th1, Th2, Th17, Antibodies)?	Murine	[48,49]
Blastomyces	Attenuated mutant	Whole	Th1, Th17	Murine	
Candida	Agglutinin-like sequence adhesins (Als1p/Als3p)/ rAls3p-N (NDV-3)*	Recombinant/subunit	Th1, Th17, Antibodies	Murine/Simian/ Phase I clinical trial	[10,50•]
Candida	Hyr1p	Recombinant/subunit	Antibodies	Murine	[51]
Candida	Secreted aspartyl proteinase protein, Sap2p PEV-7*	Recombinant truncated	Antibodies	Murine/Phase I clinical trial	[52]
Candida Cryptococcus	Laminaran	Subunit (algal β-glucan based)	Antibodies	Murine	[53,54]
Candida	Mannan linked to human serum albumin	Subunit (Candida mannan)	Th1/Antibodies	Rabbit	[55]
Candida	Live-attenuated	Genetically engineered	T cells	Murine	[56]
Candida	Fba peptide	Subunit	Antibodies	Murine	[57]
Coccidiodes	Attenuated mutant	Whole	Th1, Th17, Th2?	Murine	[8]
Coccidiodes	T-cell epitopes Antigen 2/proline rich Ag (Ag2/PRA)	Recombinant	Th1, Th17, Th2?	Murine Simian	[19•,58]
Cryptococcus	Glucuronoxylomannann (GXM) capsule Galactoxylomannan-protein	Subunit	Antibodies?	Murine	[59]
Cryptococcus	Peptide Mimotopes of GXM capsule (P13)-linked to Tetanus or Diphtheria toxoid	Subunit/Recombinant	Antibodies	Murine	[60]
Cryptococcus	CneF (culture filtrate Ags), Mannoprotein	Subunit/Recombinant	Th1, Antibodies	Murine	[61]
Histoplasma	Live, heat-killed	Whole	Th1, Th17	Murine	[2,6••]
Histoplasma	Cell wall membrane fractions/HSP	Subunit	Th1	Murine	[17,62]
Paracoccidioides	rPb27	Recombinant	Antibodies	Murine	[21]
Paracoccidiodes	HSP60	Recombinant	Th1	Murine	[22]
Paracoccidiodes	P10 (peptide)	Subunit	Th1	Murine	[20]
Pneumocystis	55 kDa DNA/p-55	Recombinant	Partial, Antibodies?	Murine	[15,16]
Pneumocystis	Kexin	Recombinant/subunit	Antibodies, $CD8^+$ T cells	Murine	[14]

* Under study in human clinical trials.

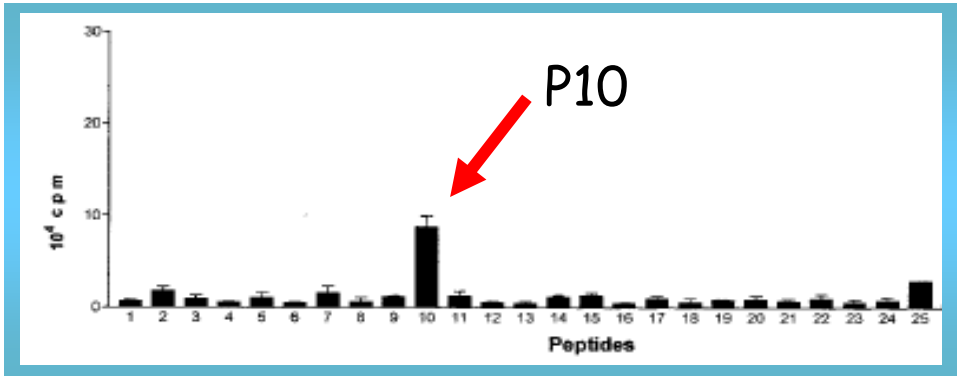
Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Immunology

Vaccine immunity against fungal infections

Som G Nanjappa[1] and Bruce S Klein[1,2,3]

Paracoccidioidomycosis – gp43

- Mapping of gp43: T cells from mice with different H-2: T epitope = P10 peptide (Taborda et al, 1998)



- Immunization of mice with gp43 or P10 conferred protection against experimental paracoccidioidomycosis (Taborda et al., 1998; Travassos et al., 2004)

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2006, p. 2814–2819 Vol. 50, No. 8
0066-4804/06/$08.00+0 doi:10.1128/AAC.00220-06

NOTES

Peptide Immunization as an Adjuvant to Chemotherapy in Mice Challenged Intratracheally with Virulent Yeast Cells of *Paracoccidioides brasiliensis*

A. F. Marques,[1] M. B. da Silva,[1] M. A. P. Juliano,[2] L. R. Travassos,[3] and C. P. Taborda[1]*

Institute of Biomedical Sciences, Department of Microbiology, University of São Paulo, São Paulo, SP, Brazil[1]; and Departments of Biophysics[2] and Microbiology, Immunology and Parasitology,[3] Federal University of São Paulo, São Paulo, SP, Brazil

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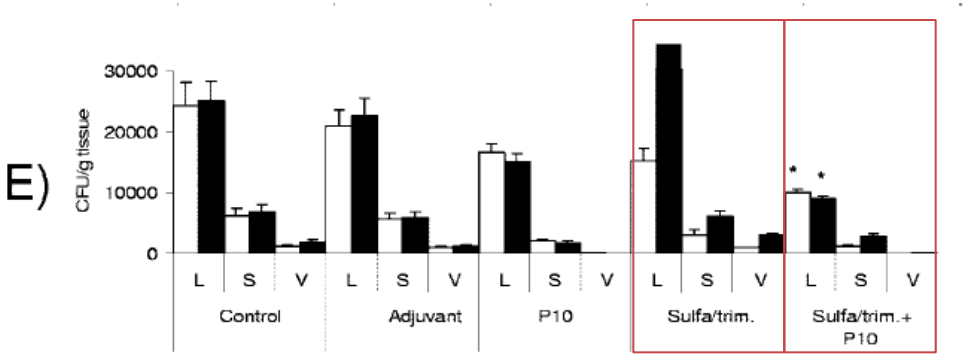

FIG. 2. Protocol 2 (treatment started 30 days after infection). CFU in organs from mice infected intratracheally with 3×10^5 yeast cells and subjected to antifungal treatment combined or not with P10 immunization were determined. Mice were sacrificed after 60 (□) and 120 (■) days of infection. Control mice were inoculated with phosphate-buffered saline, adjuvant-treated control mice were inoculated with CFA or IFA, and P10-treated mice were immunized with peptide. All groups of mice were infected with the same number of yeast cells. Experiments were carried out in triplicate. Each bar represents the average counts and standard deviations in organs from 5 to 10 animals in each group. L, lung; S, spleen; V, liver. *, significant differences between the combined treatment and both P10 vaccine alone and drug treatment alone ($P < 0.05$).

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Microbes and Infection 10 (2008) 1251–1258

www.elsevier.com/locate/micinf

Original article

Additive effect of P10 immunization and chemotherapy in anergic mice challenged intratracheally with virulent yeasts of *Paracoccidioides brasiliensis*

Alexandre F. Marques [a], Marcelo B. da Silva [a], Maria A.P. Juliano [b], Julian E. Munhõz [a], Luiz R. Travassos [c], Carlos P. Taborda [a,*]

[a] *Institute of Biomedical Sciences, Department of Microbiology, University of São Paulo, São Paulo, SP, Brazil*
[b] *Department of Biophysics, Federal University of São Paulo, São Paulo, SP, Brazil*
[c] *Department Microbiology, Immunology and Parasitology, Federal University of São Paulo, São Paulo, SP, Brazil*

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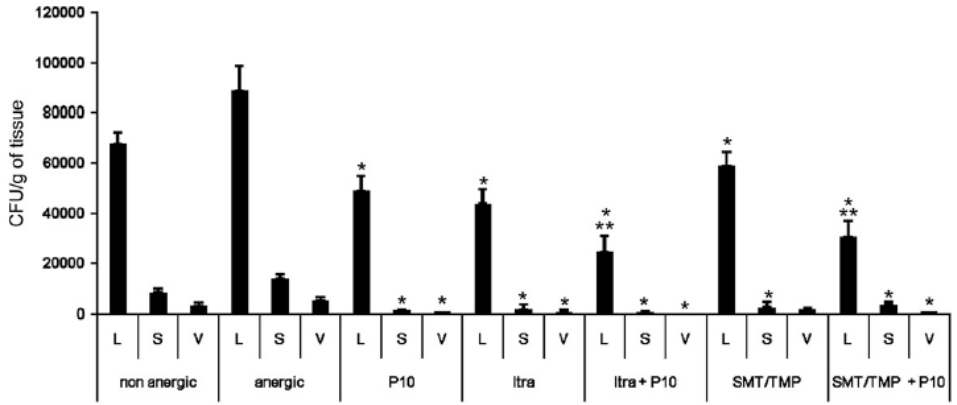
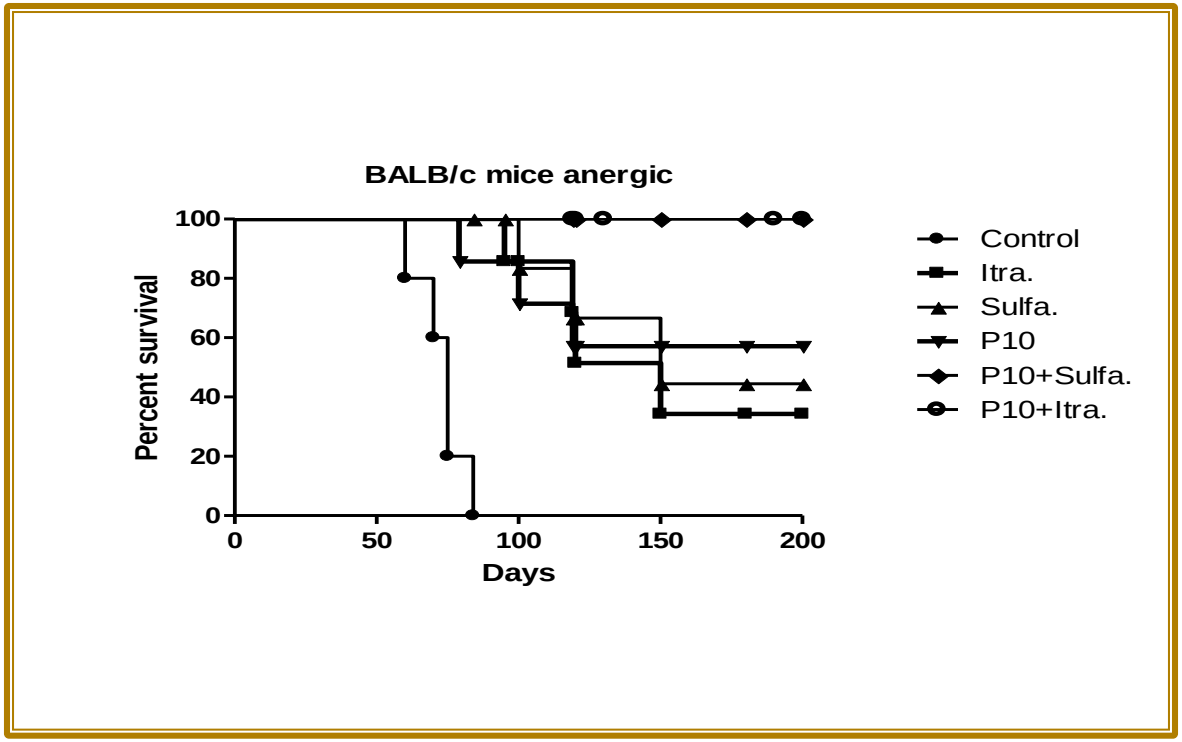


Fig. 2. Colony forming units from organs of infected mice. Colony forming units (CFU) from lungs (L), spleen (S) and liver (V) of Balb/c mice infected intratracheally with 3×10^5 yeast cells of *P. brasiliensis* Pb18 (non-anergic) or infected and treated with dexamethasone (anergic). Groups of anergic animals were also submitted to P10 immunization (P10), itraconazole (Itra) or sulfamethoxazole/trimethoprim (SMT/TMP) treatment, or the combined P10 immunization and chemotherapy (itraconazole + P10 and SMT/TMP + P10). Bars represent the CFU means and standard deviations from organs of five to 10 animals in each group. * significant ($p < 0.05$) difference in relation to the group of mice infected and treated only with dexamethasone in drinking water. ** significant ($p < 0.05$) differences between the combined treatment of P10 and antifungal drug, and the individual treatments with each agent.

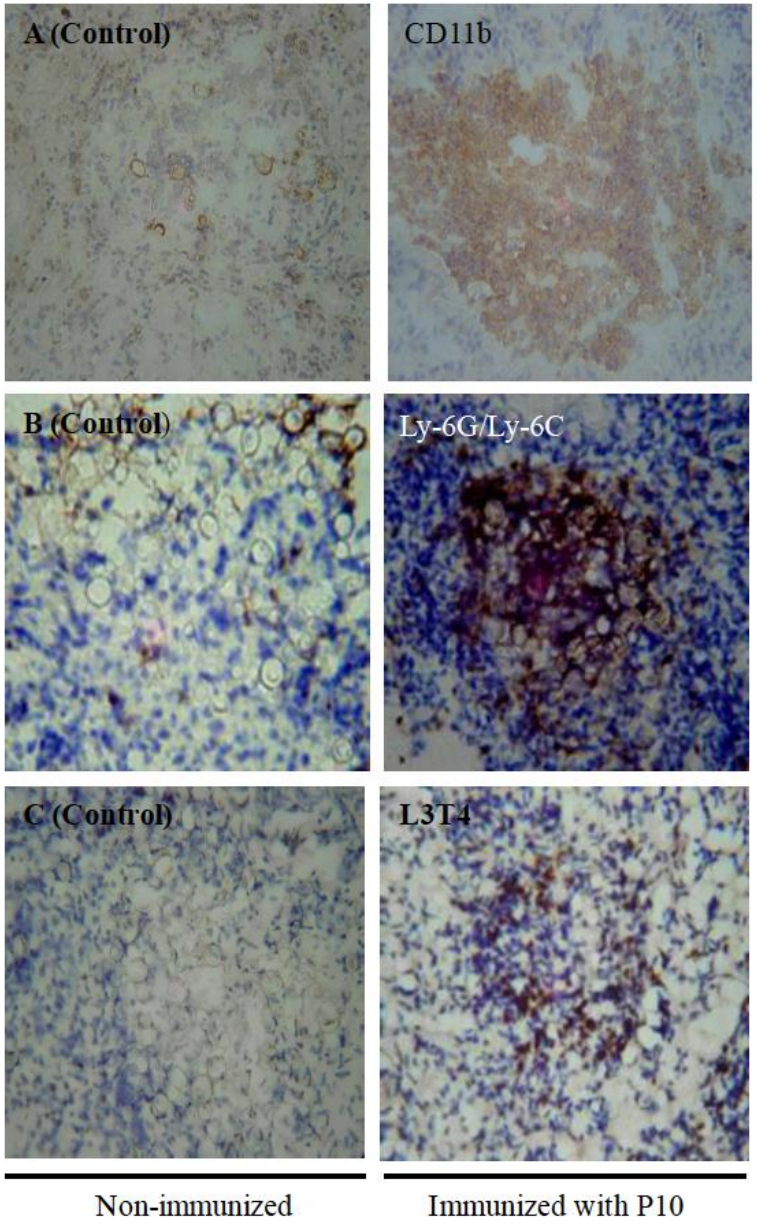
Mycopathologia (2014) 178:177–188
DOI 10.1007/s11046-014-9801-1

Immunization with P10 Peptide Increases Specific Immunity and Protects Immunosuppressed BALB/c Mice Infected with Virulent Yeasts of Paracoccidioides brasiliensis

Julián E. Muñoz · Vinicius D. Luft · Juliana Amorim · Adriana Magalhães · Luciana Thomaz · Joshua D. Nosanchuk · Luiz R. Travassos · Carlos P. Taborda



Survival curve of anergic BALB/c infected it with $3x10^5$ cells of Pb 18 during a period of 200 days. There were significant differences between groups of animals immunized with P10 and non-immunized. Values are significant at $p < 0.0001$



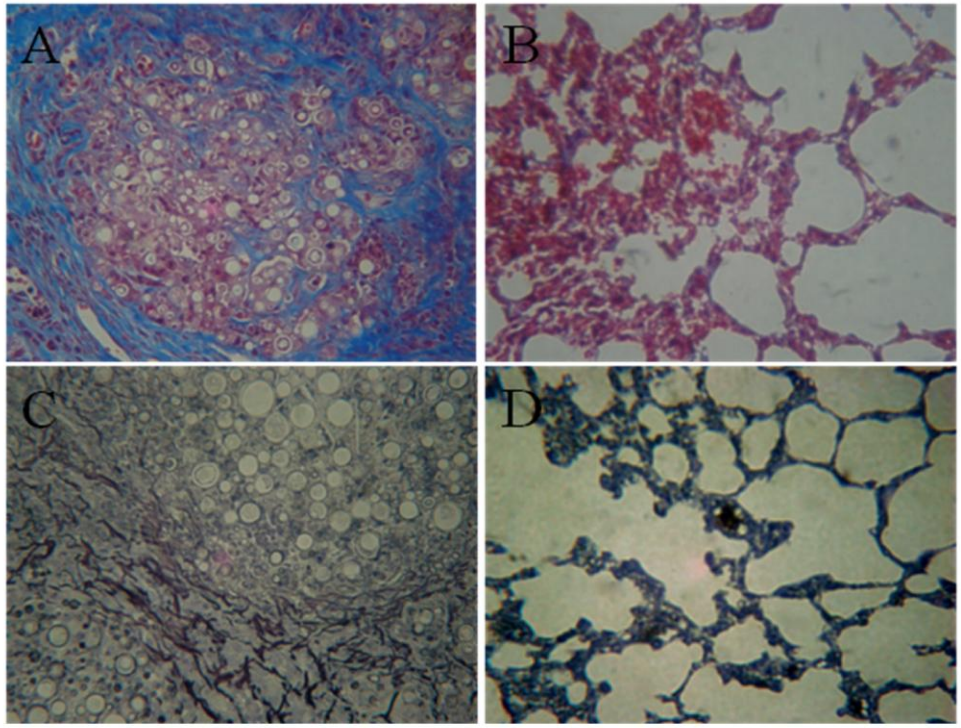
expressed at varying levels on granulocytes, macrophages, myeloid-derived dendritic cells, natural killer cells, microglia, and B-1 B lymphocytes.

recognizes granulocytes (neutrophils and eosinophils) and monocytes

CD11b+, Ly-6G/Ly-6C+ and L3T4+ cells in the lung tissues from immunosuppressed and infected BALB/c mice. Tissue sections were obtained 60 days after infection with $3x10^5$ yeast cells of *P. brasiliensis*. (A) CD11b+ cells in lung tissue of control group and immunized mice with P10. (B) Ly-6G/Ly-6C+ cells in lung tissue of control group and immunized mice with P10. (C) L3T4+ cells in lung tissue of control group and immunized mice with P10. Diaminobenzidine (DAB) was used as the peroxidase substrate to generate a brown-staining signal and the sections were counterstained with Mayer hematoxylin. Magnification, X 200.

recognizes lymphocytes

Julian E. Muñoz Henao *et al*., 2014 Mycopathologia

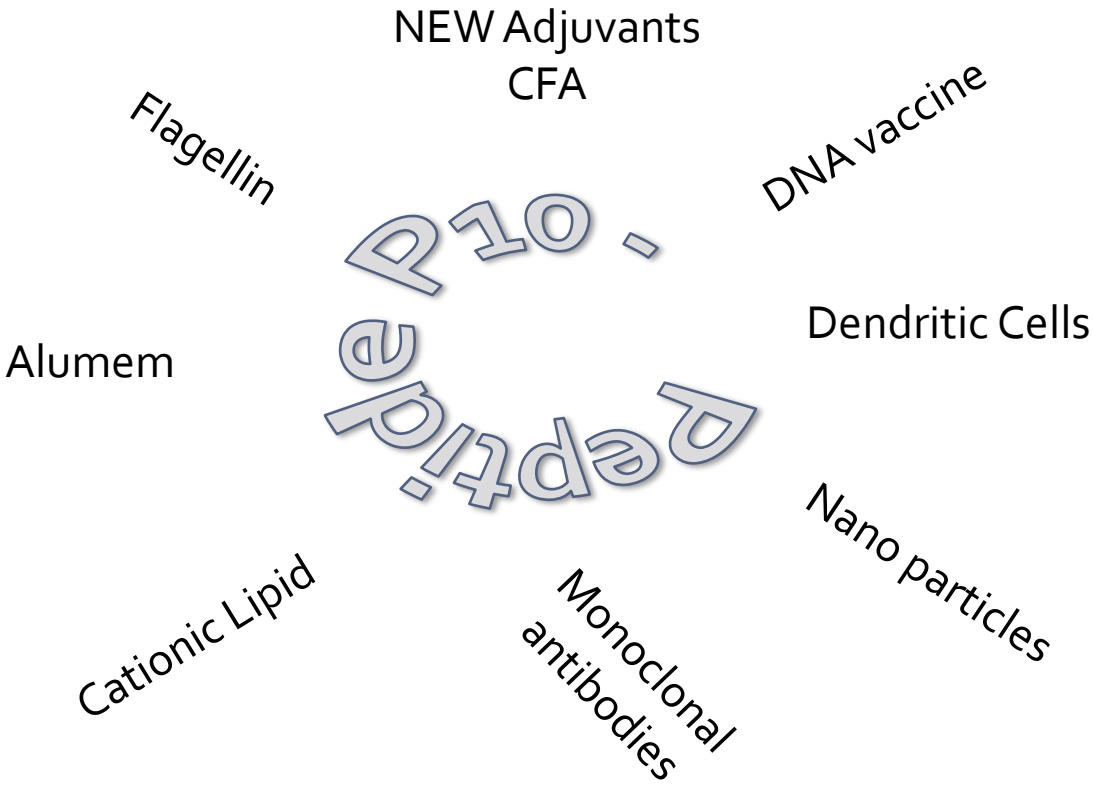


Evaluation of pulmonary fibrosis in the lungs of immunosuppressed BALB/c mice infected with 3 x 10^5 yeast cells the *P. brasiliensis* 60 days post-infection. (A, C) Untreated group. (B, D) Immunized with P10 peptide group. (A, B) Masson's Trichrome staining to detect the fibers of collagen type I and (C, D) Gomori's silver reticulin staining to detect collagen type III fibers. Magnification 100X.

Julian E. Muñoz Henao *et al*., 2014 Mycopathologia

P10 x *P. brasiliensis*

Prophylactic and Therapeutic



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0019-9567/09/$08.00+0 doi:10.1128/IAI.01470-08

Paracoccidioides brasiliensis Vaccine Formulations Based on the gp43-Derived P10 Sequence and the *Salmonella enterica* FliC Flagellin▽

Catarina J. M. Braga,[1] Glauce M. G. Rittner,[1] Julian E. Muñoz Henao,[1] Aline F. Teixeira,[1] Liliana M. Massis,[1] Maria E. Sbrogio-Almeida,[2] Carlos P. Taborda,[1] Luiz R. Travassos,[3] and Luís C. S. Ferreira[1]*

Departamento de Microbiologia, Universidade de São Paulo, São Paulo, Brazil[1]; Divisão de Desenvolvimento Tecnológico e Produção, Instituto Butantan, São Paulo, Brazil[2]; and Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal de São Paulo, São Paulo, Brazil[3]

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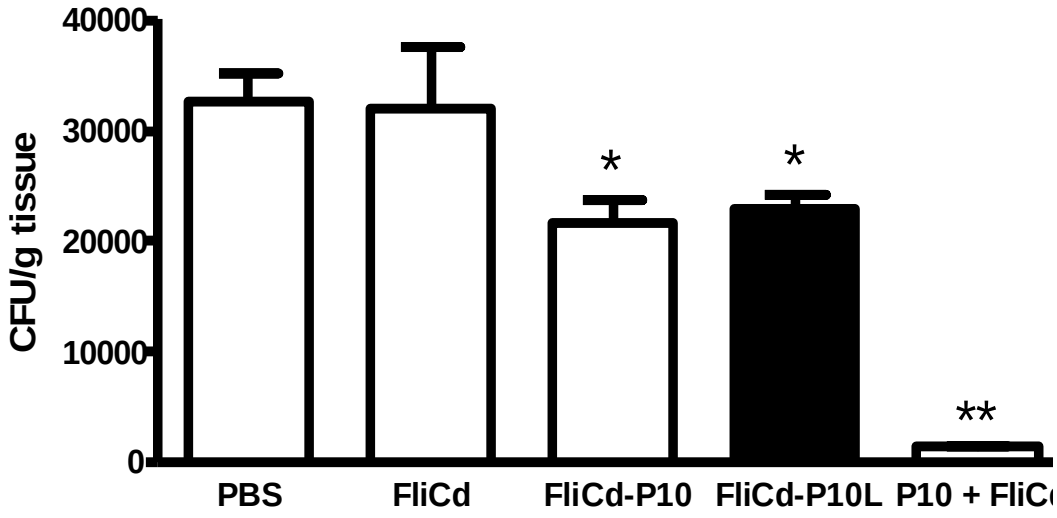

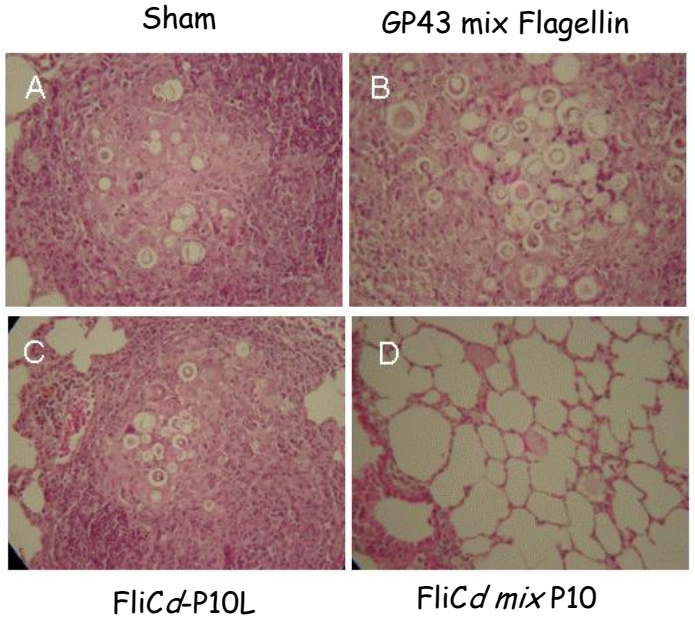

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BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2010), ••, ••–••

0007-1188/10
www.brjpharmacol.org

RESEARCH PAPER

Poly(lactic acid-glycolic acid) nanoparticles markedly improve immunological protection provided by peptide P10 against murine paracoccidioidomycosis

André C Amaral[1,2], Alexandre F Marques[3]*, Julián E Muñoz[3], Anamélia L Bocca[1], Andreza R Simioni[4], Antonio C Tedesco[4], Paulo C Morais[5], Luiz R Travassos[6], Carlos P Taborda[3] and Maria Sueli S Felipe[1]

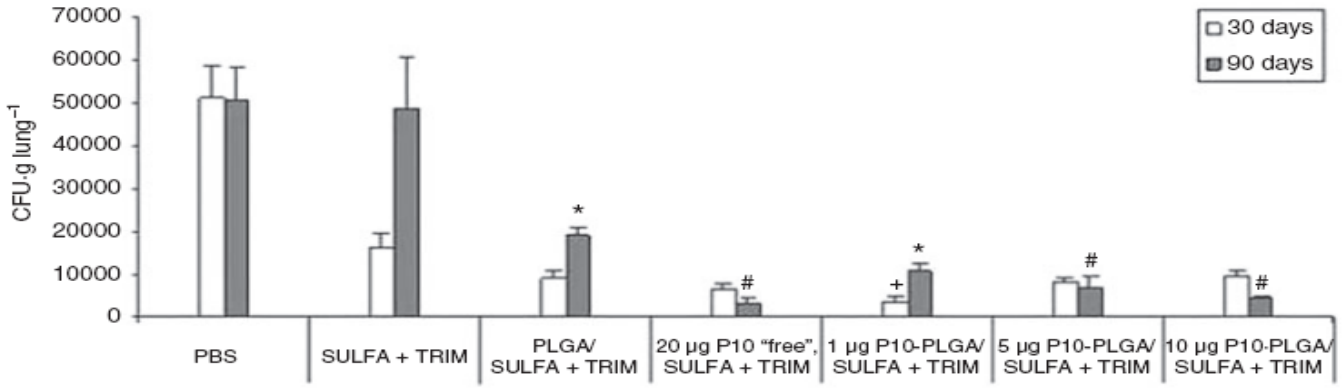

Figure 1 Fungal burden recovery assessed by colony-forming units [CFU (g·lung·tissue)$^{-1}$] in mice infected with *P. brasiliensis* Pb18 and subjected to a combined therapy of sulfamethoxazole/trimethoprim (Sulfa + Trim; 15 mg·kg^{-1} and 3 mg·kg^{-1} respectively) and either 20 µg·50 µL^{-1} P10 peptide solubilized in Freund's adjuvant ('free') or P10 peptide (1, 5 or 10 µg·50 µL^{-1}) entrapped within PLGA. Each bar represents the average CFU (g·tissue)$^{-1}$ with standard deviations. After 30 days of treatment, 1 µg·50 µL^{-1} of P10-PLGA/Sulfa + Trim yielded the best response (lowest fungal CFU recovery) of all groups (+$P < 0.05$). After 90 days of treatment, no significant differences were found between the responses of the 'free' P10 (20 µg·50 µL^{-1}) and the P10-PLGA (5–10 µg·50 µL^{-1})-treated groups (marked with #). At day 90, a significantly lower number of fungal cells were recovered from mice treated with 1 µg·50 µL^{-1} P10-PLGA compared with the PLGA alone treated group (*$P < 0.05$). PLGA, poly(lactic acid-glycolic acid).

frontiers in MICROBIOLOGY

ORIGINAL RESEARCH ARTICLE
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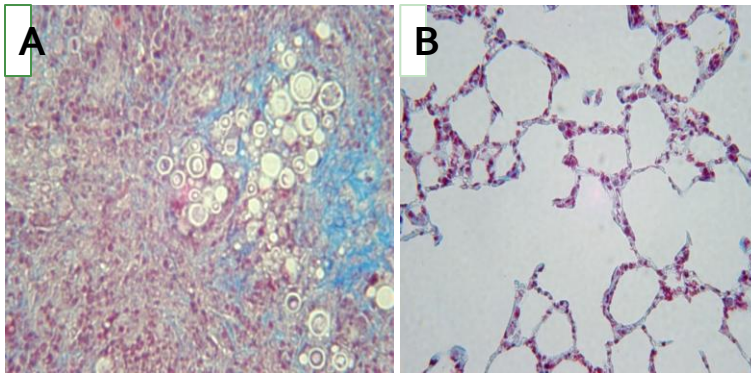
The role of adjuvants in therapeutic protection against paracoccidioidomycosis after immunization with the P10 peptide

*Oriana Mayorga[1], Julian E. Muñoz[1], Nilton Lincopan[1,2], Aline F. Teixeira[1], Luis C. S. Ferreira[1], Luiz R. Travassos[3] and Carlos P. Taborda[1,4]**

Cationic Lipid/P10 complex adjuvanted



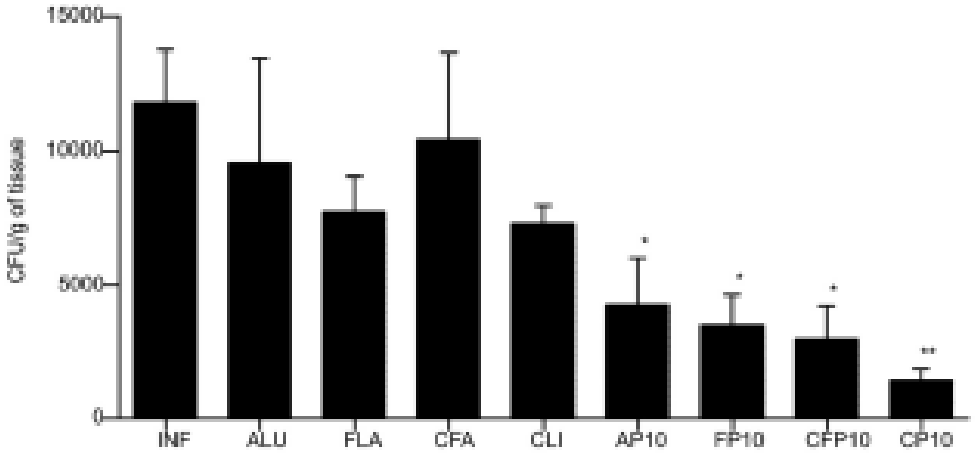


FIGURE 1 | Colony forming units (CFU) from lungs of BALB/c mice infected intratracheally with 3 × 10^5 yeast cells of Pb18 and immunized at 30, 37, and 44 days after infection with the different adjuvants with or without P10. Animals were sacrificed after 60 days of infection. Control animals were infected by not immunized (IFN). The adjuvants used were: aluminum hydroxide alone (ALU) or with P10 (AP10), FliC flagellin alone (FLA) or with P10 (FP10), complete Freund's adjuvant alone (CFA) or with P10 (CF10), and cationic lipid alone (CLI) or with P10 (CP10). Significant difference $*p < 0.05$, $**p < 0.01$.

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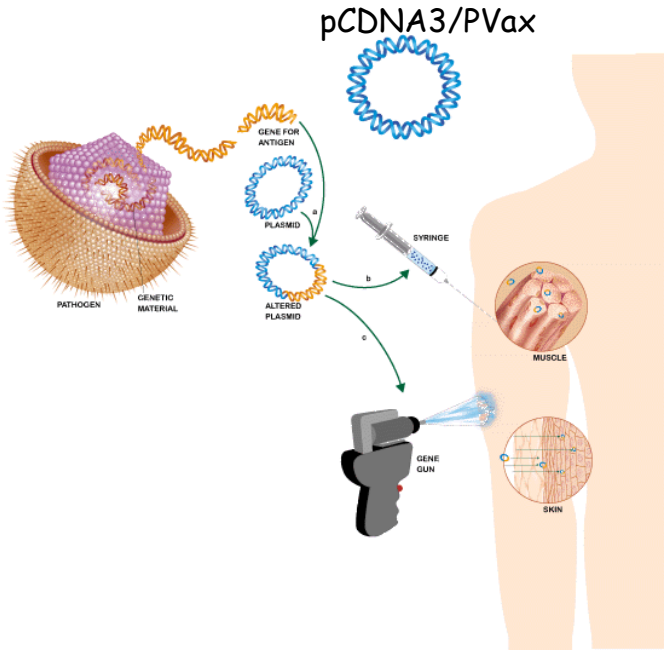
PLoS NEGLECTED TROPICAL DISEASES

Therapeutic DNA Vaccine Encoding Peptide P10 against Experimental Paracoccidioidomycosis

Glauce M. G. Rittner[1], Julián E. Muñoz[1], Alexandre F. Marques[1], Joshua D. Nosanchuk[2], Carlos P. Taborda[1,3], Luiz R. Travassos[4]*



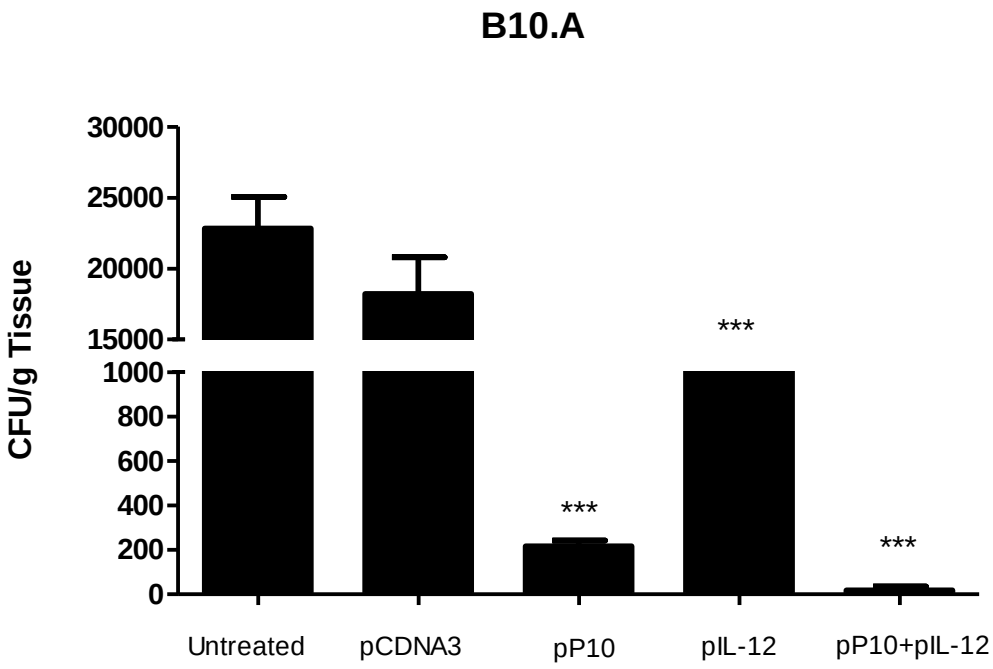


Figure: Therapeutic treatment of experimental PCM. Gene therapy started 30 days after infection and mice were sacrificed 5 months after infection. Immunizations were done every month during three months. CFUs were counted in lungs of B10.A mice infected intratracheally with 3 x10^5 yeast cells and subjected to vaccine of vectors containing the insert P10 (pP10) or IL-12 (pIL-12). Control mice were inoculated with PBS and vaccinated with vector without insert. Each bar represents the average counts and standard deviations of CFU in lungs from 5 to 10 animals in each group. Experiments were carried out in triplicate. *, significant difference between the vector with insert and vector without P10 or IL-12 insert ($p \leq 0.05$).

Generation of memory and regulatory T Cells in BALB/c mice immunized with plasmid DNA encoding the P10 peptide of *Paracoccidioides brasiliensis*

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Microbes and Infection 15 (2013) 181–191

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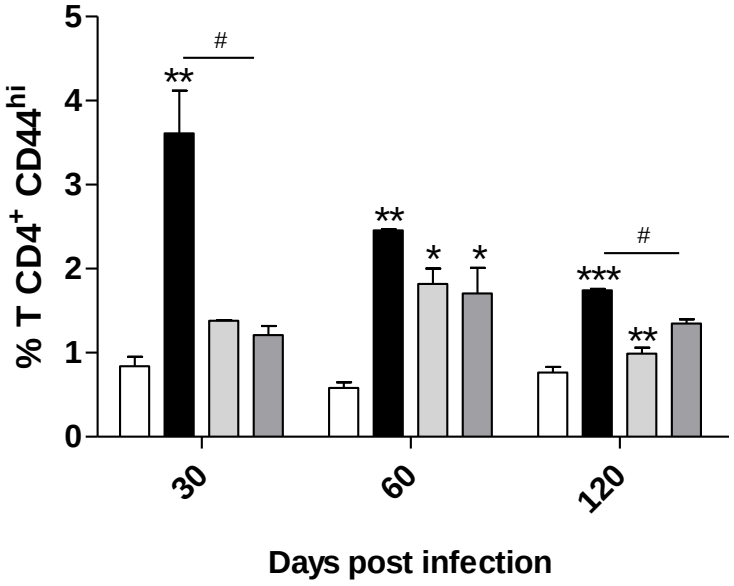
Original article

DNA vaccine encoding peptide P10 against experimental paracoccidioidomycosis induces long-term protection in presence of regulatory T cells

Juliana de Amorim [a], Adriana Magalhães [a], Julián Esteban Muñoz [a], Glauce M.G. Rittner [a], Joshua D. Nosanchuk [b,c], Luiz R. Travassos [d], Carlos P. Taborda [a,e,*]



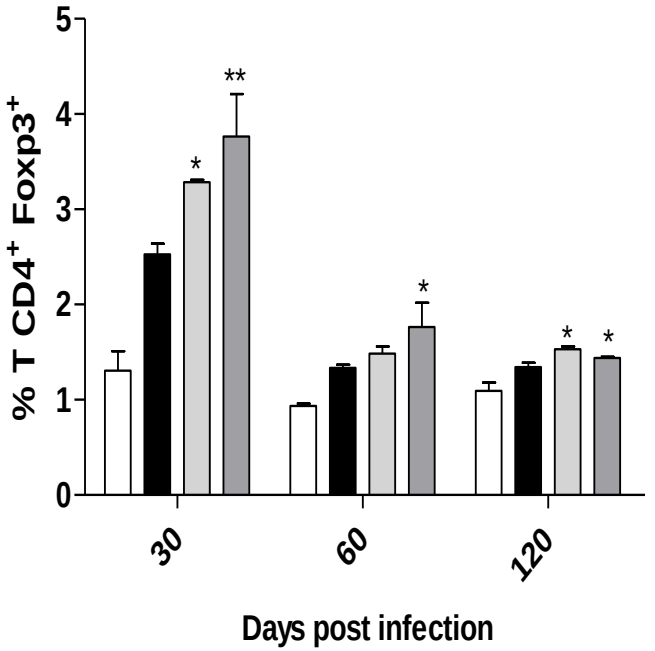


Figure - Percentage of pulmonary lymphocytes with memory or regulatory phenotype ($CD4^+CD44^{hi}$ or $CD4^+$ $Foxp3^+$, respectively) of mice immunized with the plasmid DNA encoding the P10 peptide and challenged – pP10 (black bars), mice immunized with the empty vector and challenged – pcDNA3 (light gray bars) and mice not immunized and infected with *P. brasiliensis* (dark gray bars). Age-matched controls not immunized and not infected are shown in white bars.

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Prophylactic and Therapeutic Vaccination Using Dendritic Cells Primed with Peptide 10 Derived from the 43-Kilodalton Glycoprotein of *Paracoccidioides brasiliensis*

A. Magalhães,[a] K. S. Ferreira,[b] S. R. Almeida,[c] J. D. Nosanchuk,[d] L. R. Travassos,[e] and C. P. Taborda[a,f]

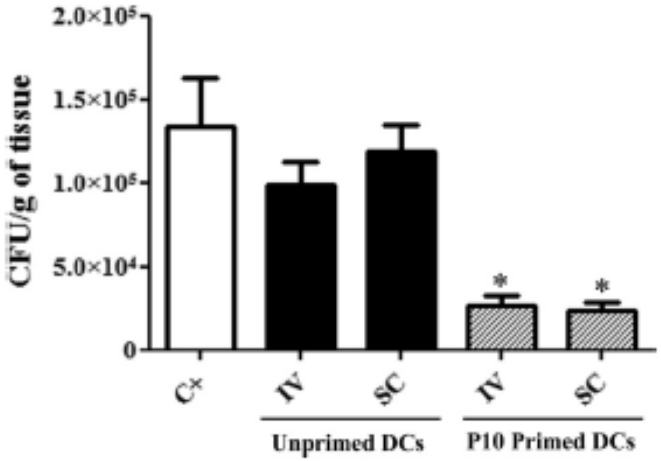


FIG 2 Lung CFU: therapeutic protocol. The results are from two independent experiments. Each group from each experiment ($n = 5$) was infected i.t. with 3×10^5 yeast cells. After 30 days, groups of mice received either nonprimed dendritic cells (DCs) or P10-primed DCs via either an intravenous (IV) or subcutaneous (SC) route. A second identical immunization was administered 7 days later. The control group (C+) was not treated. Mice were sacrificed at day 45 after infection. *, significant difference ($P < 0.05$) compared with the control and unprimed DCs.

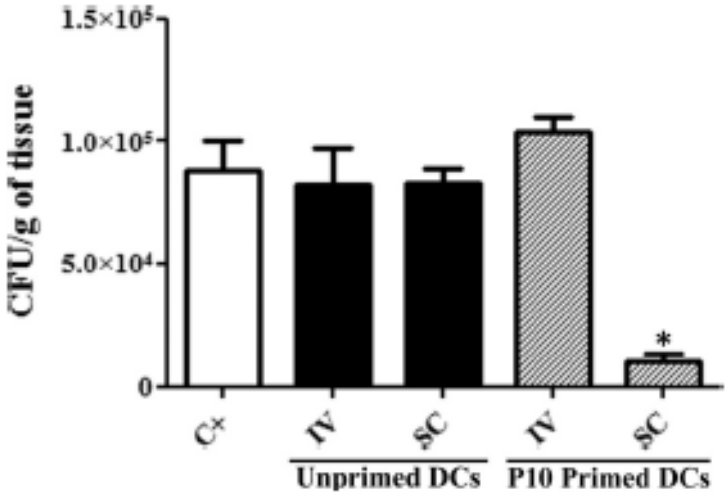
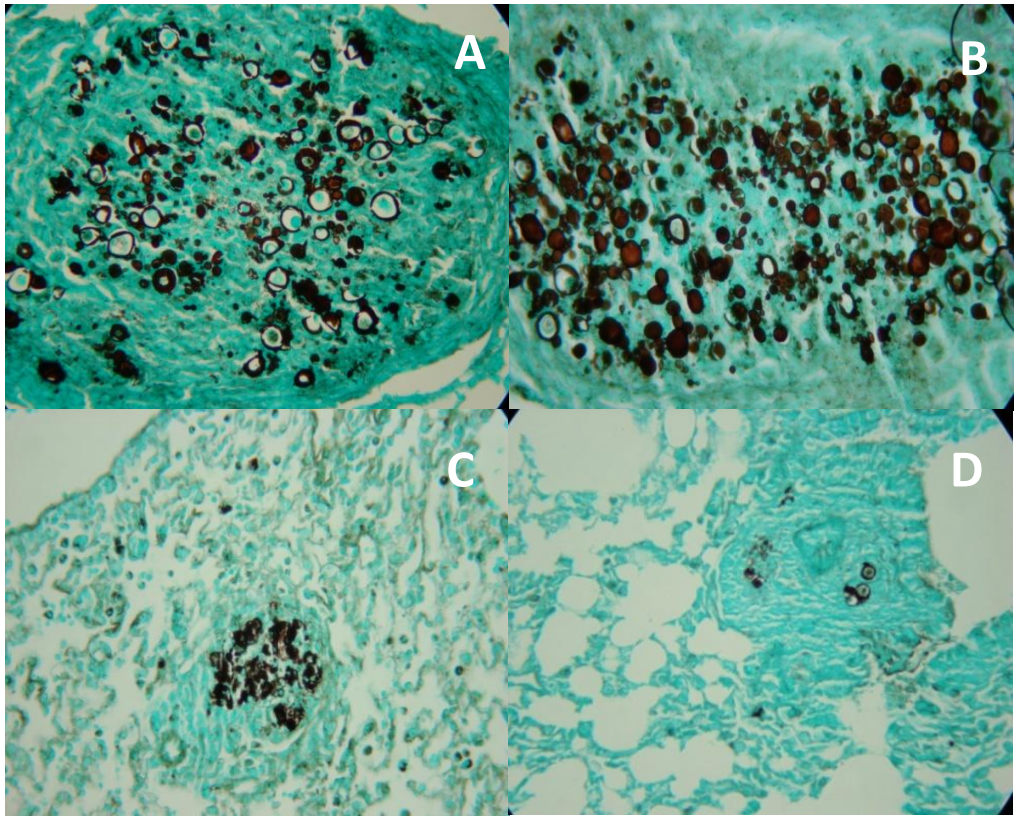


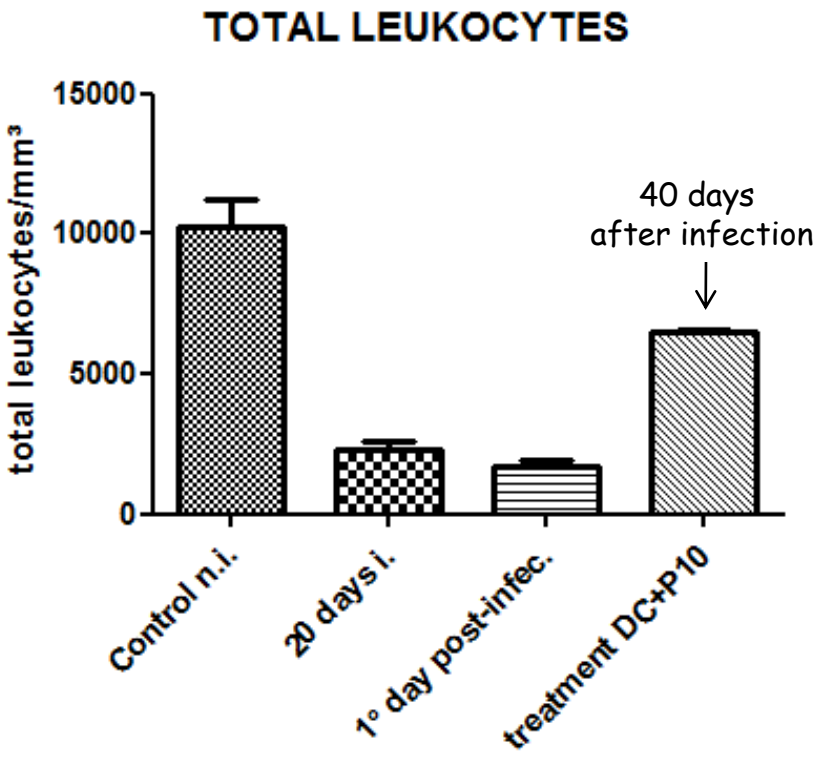
FIG 3 Lung CFU: prophylactic protocol. The results are from two independent experiments. The groups of treated mice ($n = 5$ per experiment) received either unprimed dendritic cells (DC) or P10-primed DCs via either intravenous (IV) or subcutaneous (SC) route 24 h before the mice were infected i.t. with 3×10^5 yeast cells. The control (C+) group received PBS 1 day prior to infection. Mice were sacrificed 30 days after infection. *, significant difference ($P < 0.001$) compared with the control.

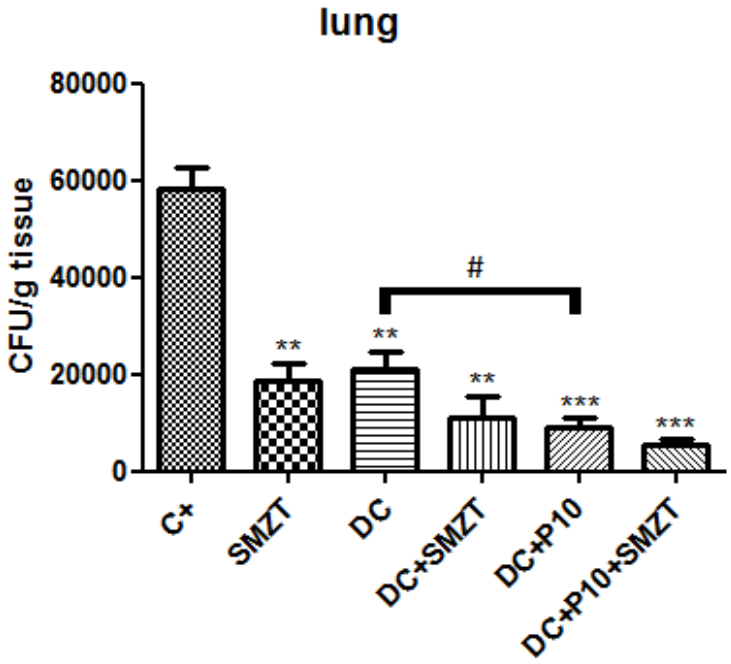
Histopathology of lung from group of 30 day-therapeutic protocol



Histopathology of lung samples from group of 30 day-therapeutic protocol. Animals were infected and 30 days after, received two doses of the vaccine with an interval of seven days. Mice were sacrificed seven days after the second dose. **A.** infected and untreated; **B.** received unprimed DCs; **C**. received P10-primed DCs by the intravenous route and **D**. the same as C by the subcutaneous route (400X magnification).

Therapeutic vaccination using DCs primed with P10 in anergic mice





Da Silva, L. B. R et al., submitted 2016

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Advance Access Publication Date: 12 June 2014
Original Article

Original Article

Radiochemical pharmacokinetic profile of P10 peptide with antifungal properties

Bluma L. Faintuch[1,*], Erica A. Oliveira[1], Julian E. Munõz[2], Luiz R. Travassos[3] and Carlos P. Taborda[2,4]

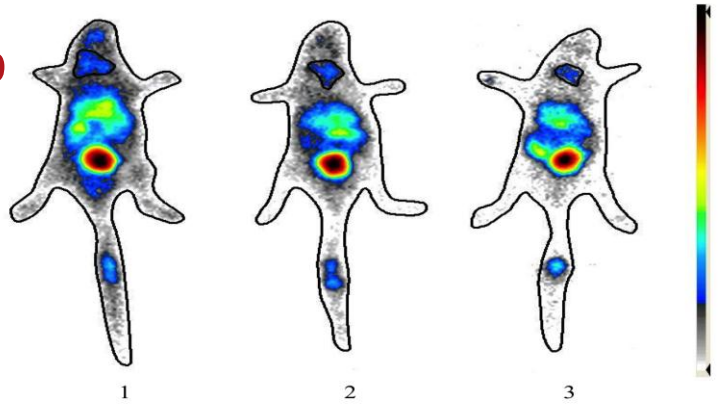


Figure 5 - Scintilographic image obtained in a Gamma-Camara. (1) Image acquired 30 min post-injection (p.i.); (2) at 60 min p.i.; and (3) at 120 min p.i.

Table 1 – Biodistribution of the radiolabeled peptide in healthy mice

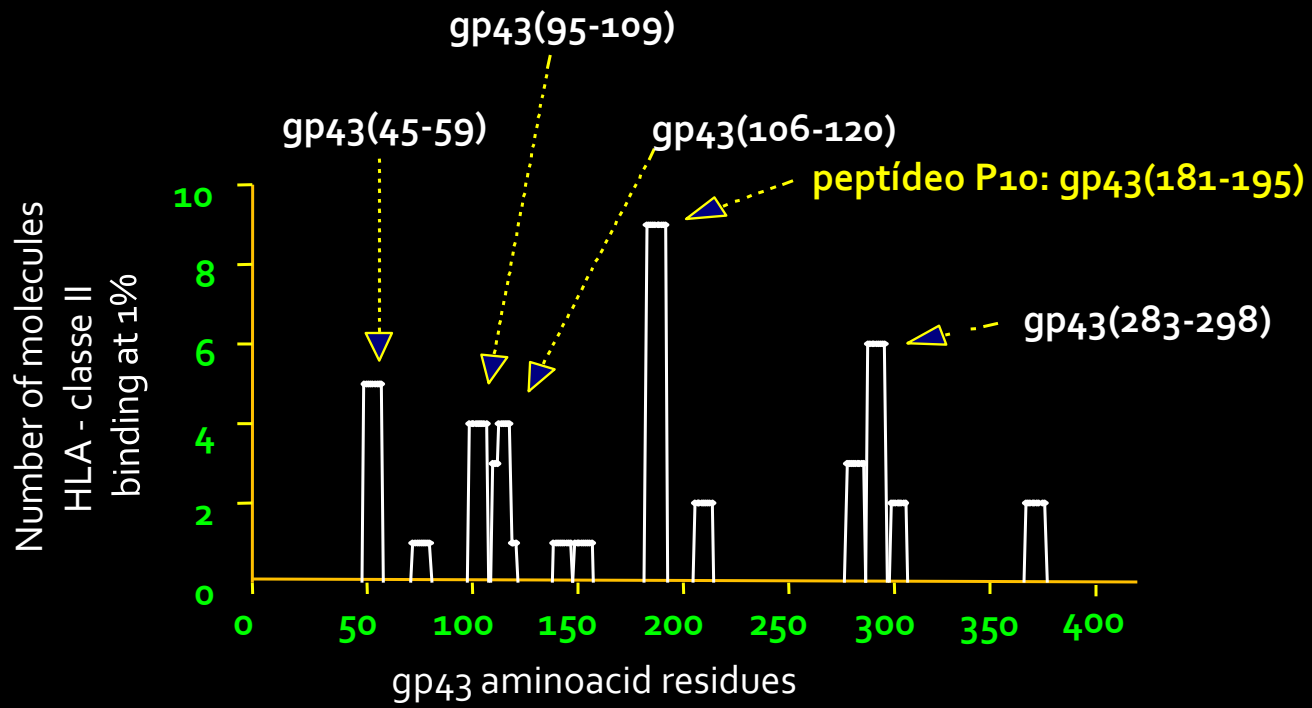
Organ/Time	5 min	30 min	1h	2h	4h	6h
Heart	2.67 ± 0.91	0.74 ± 0.37	0.37 ±0.18	0.18 ± 0.09	0.11 ± 0.01	0.11 ± 0.02
Lungs	12.07 ± 2.02	4.81 ± 2.90	5.50 ± 2.85	3.43 ± 2.88	1.17 ± 0.27	1.05 ± 0.19
Spleen	2.09 ± 0.29	2.80 ± 2.23	3.95 ± 2.50	2.07 ± 1.84	3.21 ± 2.51	3.58 ± 0.49
Stomach	4.14 ± 1.59	3.13 ± 0.48	1.72 ± 0.57	1.68 ± 0.55	1.10 ± 0.26	0.97 ± 0.42
Pancreas	1.72 ± 0.34	0.85 ± 0.47	0.42 ± 0.24	0.20 ± 0.08	0.12 ± 0.02	0.11 ± 0.03
Brain	0.27 ± 0.08	0.11 ± 0.05	0.05 ± 0.03	0.03 ± 0.02	0.02 ± 0.00	0.03 ± 0.01

Data are expressed as means ± SD (n = 5). The radioactivity in the stomach was evaluated after completely removing the luminal contents.

In Silico Prediction of Peptides Binding to Multiple HLA-DR Molecules Accurately Identifies Immunodominant Epitopes from gp43 of *Paracoccidioides brasiliensis* Frequently Recognized in Primary Peripheral Blood Mononuclear Cell Responses from Sensitized Individuals

Leo Kei Iwai,[1,3,5] Márcia Yoshida,[4] John Sidney,[8] Maria Aparecida Shikanai-Yasuda,[4] Anna Carla Goldberg,[1,3] Maria Aparecida Juliano,[5] Jurgen Hammer,[7] Luiz Juliano,[5] Alessandro Sette,[8] Jorge Kalil,[1,2,3] Luiz Rodolpho Travassos,[6] and Edecio Cunha-Neto[1,2,3]

TEPITOPE algorithm



Peptide	Sequence
GP_{43} (45-59)	IGGWLLLEPWISPS V $-NH_2$
GP_{43} (94-108)	TEDDFKNIAAAGLNHV-NH_2
GP_{43} (106-120)	LNHVRIPIGYWAVNP-NH_2

INFECTION AND IMMUNITY, July 2008, p. 3321–3328 Vol. 76, No. 7
0019-9567/08/$08.00+0 doi:10.1128/IAI.00349-08

The Monoclonal Antibody against the Major Diagnostic Antigen of *Paracoccidioides brasiliensis* Mediates Immune Protection in Infected BALB/c Mice Challenged Intratracheally with the Fungus▽

R. Buissa-Filho,[1] R. Puccia,[2] A. F. Marques,[1] F. A. Pinto,[1] J. E. Muñoz,[1] J. D. Nosanchuk,[3] L. R. Travassos,[2] and C. P. Taborda[1]*

Institute of Biomedical Sciences, Department of Microbiology, University of São Paulo, São Paulo, SP, Brazil[1]; Department of Microbiology, Immunology and Parasitology, Federal University of São Paulo, São Paulo, SP, Brazil[2]; and Departments of Medicine and Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York[3]

Received 17 March 2008/Returned for modification 8 April 2008/Accepted 24 April 2008

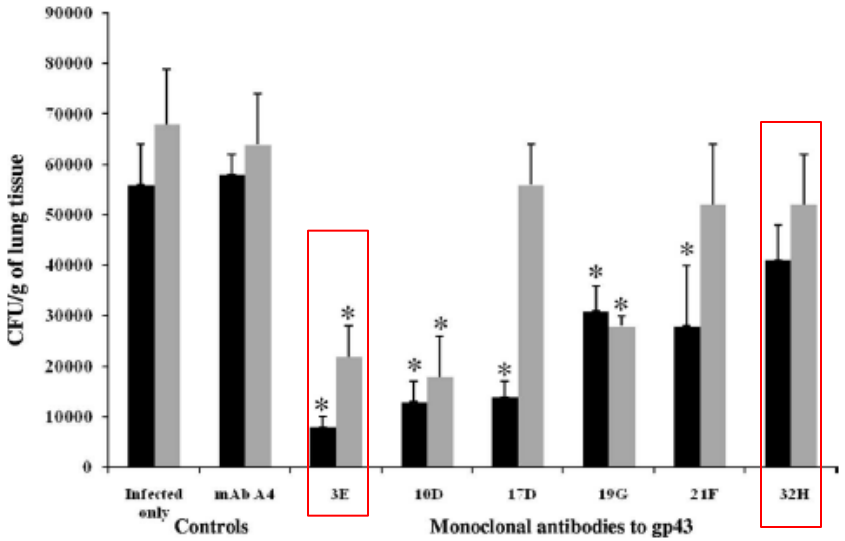


FIG. 3. Lung CFU from mice infected i.t. with 3×10^5 yeast cells and treated with MAbs against gp43 (3E, 10D, 17D, 19G, 21F, and 32H) 24 h prior to infection. Mice were sacrificed after 15 (black bars) or 30 (gray bars) days of infection. Control mice were infected and received PBS (infected only) or an irrelevant MAb (A4). Each bar represents the average count of fungi in the lung, and error bars indicate SD. *, significant difference ($P < 0.05$, determined by analysis of variance and Tukey's honestly significant difference test) relative to the PBS control.

Determination of Epitope Reactivity

NHVRIPIGYWAV(R-CONH$_2$ or COOH)

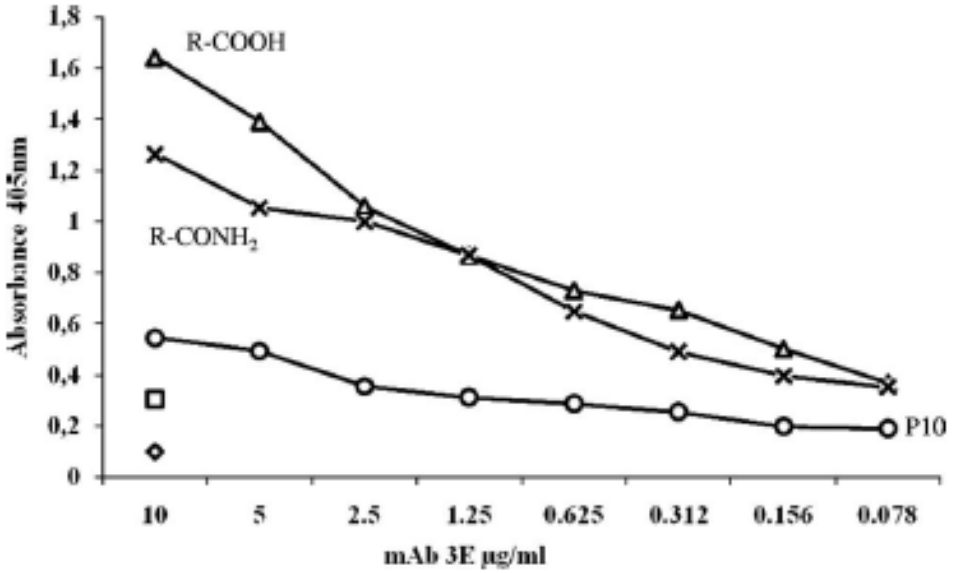
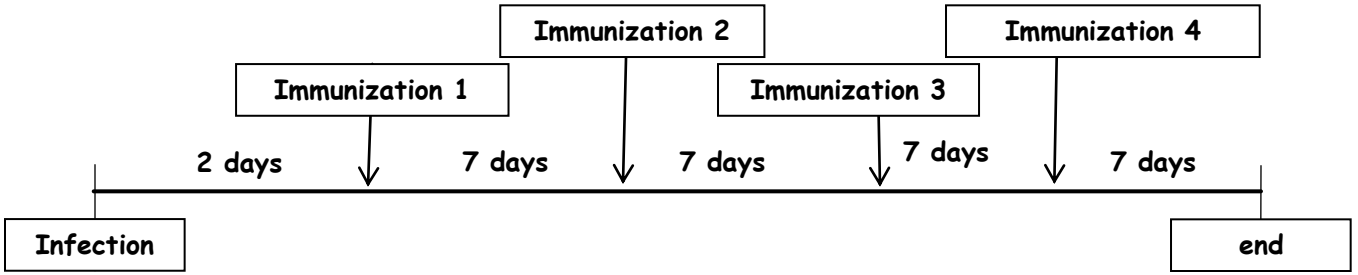
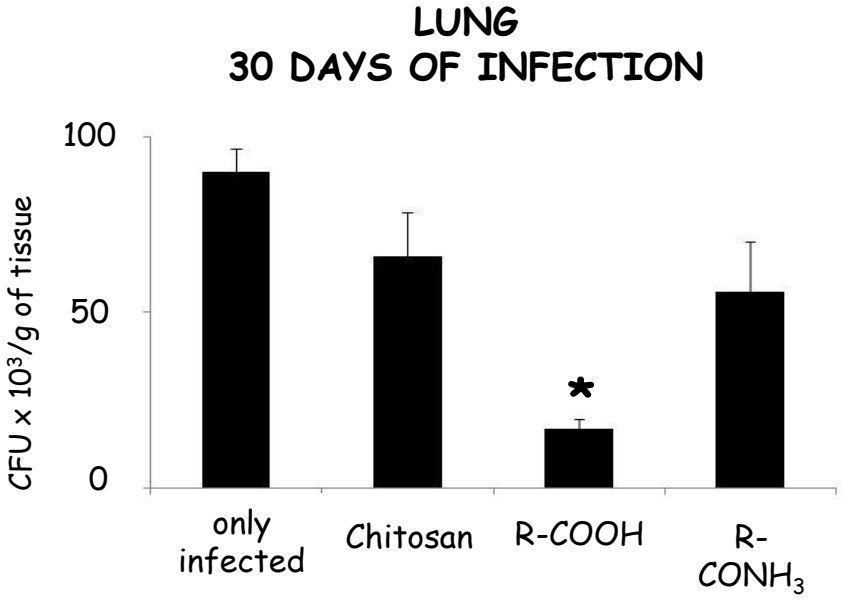
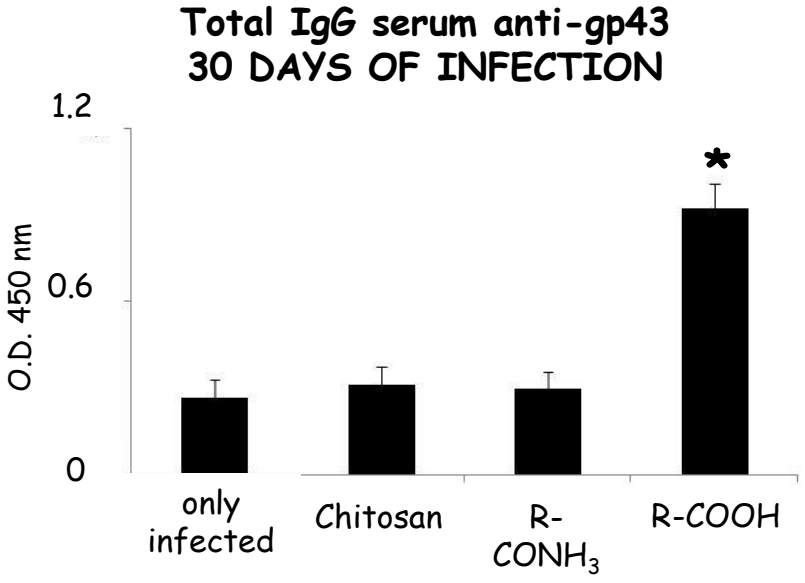


FIG. 7. Reactivity of peptides NHVRIPIGYWAV(R-CONH$_2$), -(R-COOH), and P10 (QTLAIAHTLAIRYAN) with MAb 3E by ELISA. Microtiter plates were sensitized with 100 ng of each peptide, and the reaction was developed with MAb 3E. The diamond symbol indicates the background measurement with buffer alone, and the square symbol corresponds to the reactivity of the peptides against the irrelevant antibody.

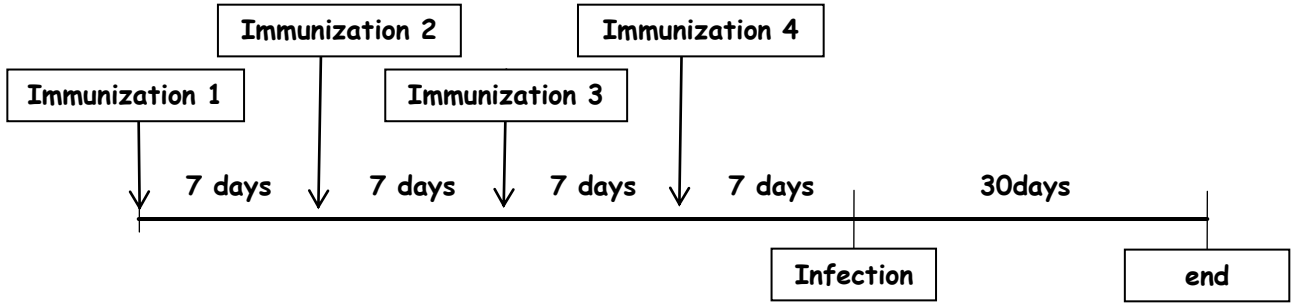
Immunization of BALB/c mice with mAb3E reactive peptide Therapeutic

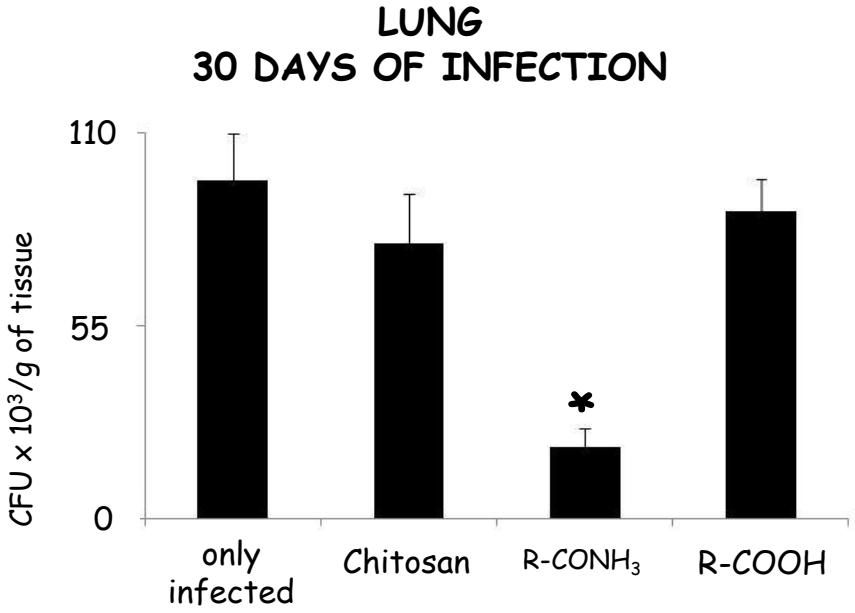


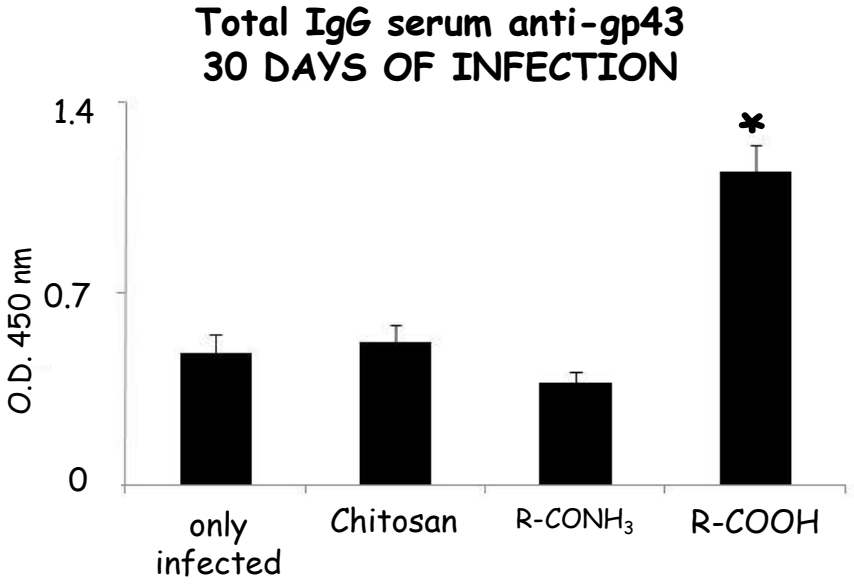




Immunization of BALB/c mice with mAb3E reactive peptide Prophilatic







Production of antibodies against glycolipids of *Paracoccidioides brasiliensis*

frontiers in Microbiology

ORIGINAL RESEARCH
published: 03 February 2016
doi: 10.3389/fmicb.2016.00074

Antibodies Against Glycolipids Enhance Antifungal Activity of Macrophages and Reduce Fungal Burden After Infection with *Paracoccidioides brasiliensis*

*Renata A. Bueno[1,2†], Luciana Thomaz[1†], Julian E. Muñoz[1], Cássia J. da Silva[1], Joshua D. Nosanchuk[3,4], Márcia R. Pinto[5], Luiz R. Travassos[6] and Carlos P. Taborda[1,2]**

Production of antibodies against glycolipids of *Paracoccidioides brasiliensis*

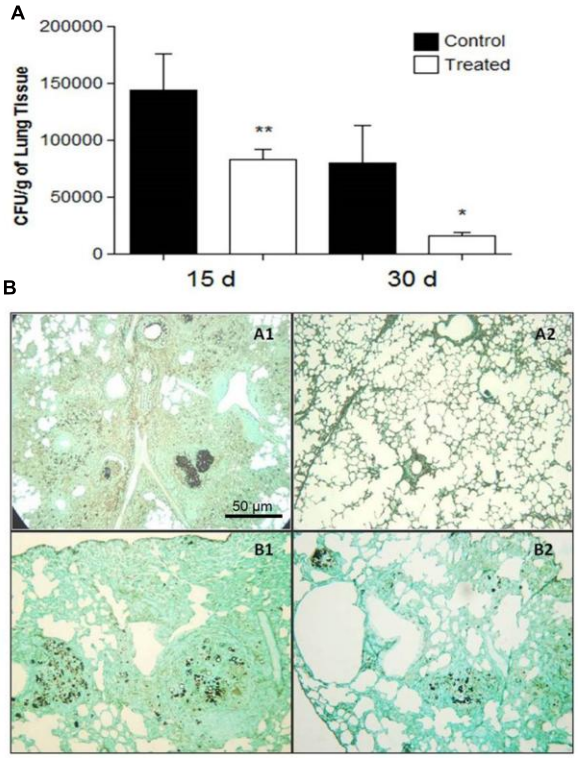


FIGURE 3 | (A) Colony forming units (CFU) in the lungs from BALB/c mice that received 1 mg of polyclonal antibodies against acidic glycolipids and 1 mg anti-BSA polyclonal antibodies (control) 24 h before infection with Pb18 and sacrificed after 15 and 30 days (prophylactic protocol). Significant values comparing the lungs of treated and control groups. $^{*}p < 0.05$, $^{**}p < 0.01$. (B) Representative lung sections: Prophylactic protocol. 24 h prior to infection, mice received polyclonal antibodies to acidic GSLs. Histopathological sections of murine lungs 15 (A1, A2) and 30 (B1, B2) days after i.t. infection. (A1, B1) Lungs from control mice; (A2, B2) Histopathological sections after i.t. infection. Photographs of sections were taken at 100× magnification.

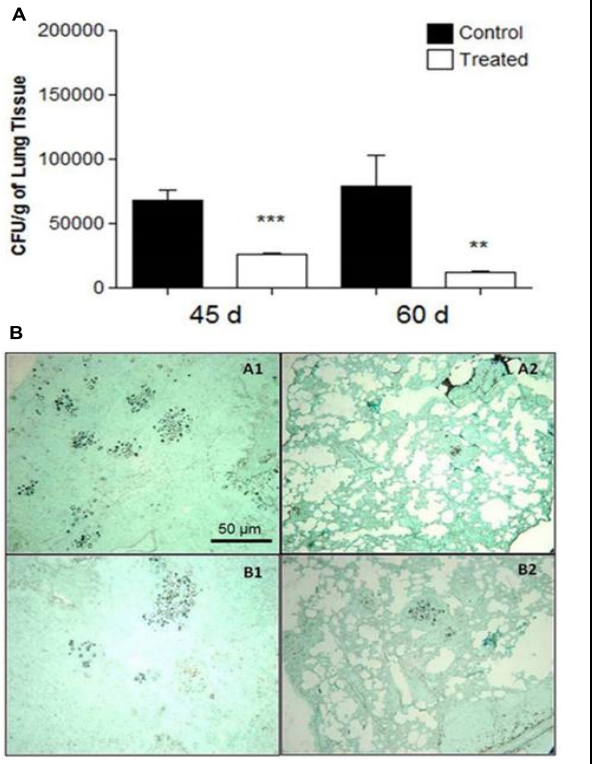


FIGURE 4 | (A) Colony forming units (CFU) in the lungs from BALB/c mice that received 1 mg of polyclonal antibodies against acidic glycolipids and 1 mg anti- BSA polyclonal antibodies (control) 30 days after infection with Pb18 and sacrificed after 45 and 60 days (therapeutic protocol). Significant values comparing the lungs of treated and control groups. $^{**}p < 0.01$, $^{***}p < 0.0001$. (B) Representative lung sections: therapeutic protocol. 3 days after i.t. infection, mice received polyclonal antibodies to acidic GSLs. Controls received polyclonal antibodies to BSA. Representative histopathological sections 45 days after i.t. infection: (A1) Lung from control mouse; (A2) Lung from treated mouse. After 60 days of i.t. infection: (B1) Control; (B2) Treated. Photographs of sections were taken at 100× magnification.

Production of antibodies against glycolipids of *Paracoccidioides brasiliensis*

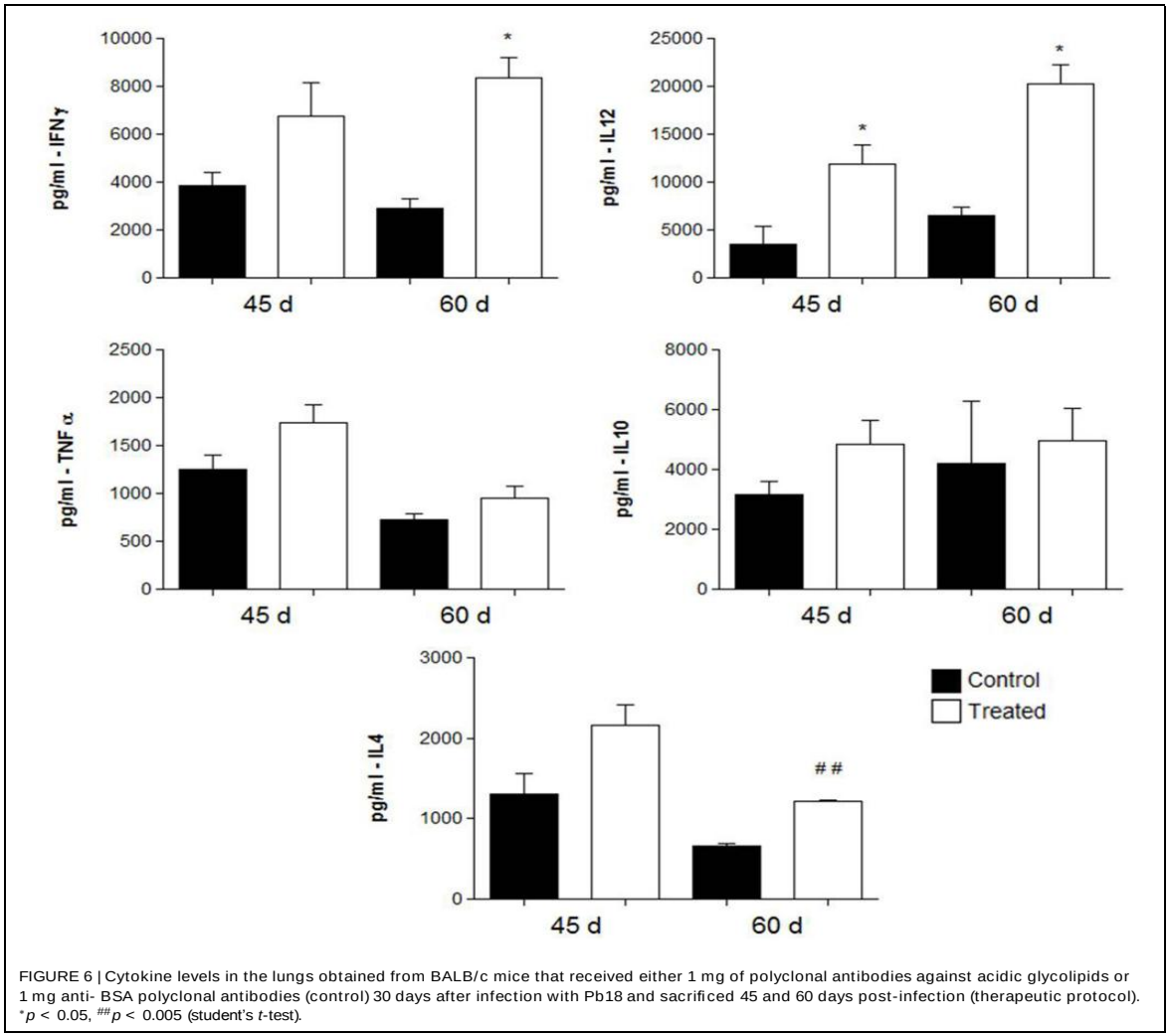
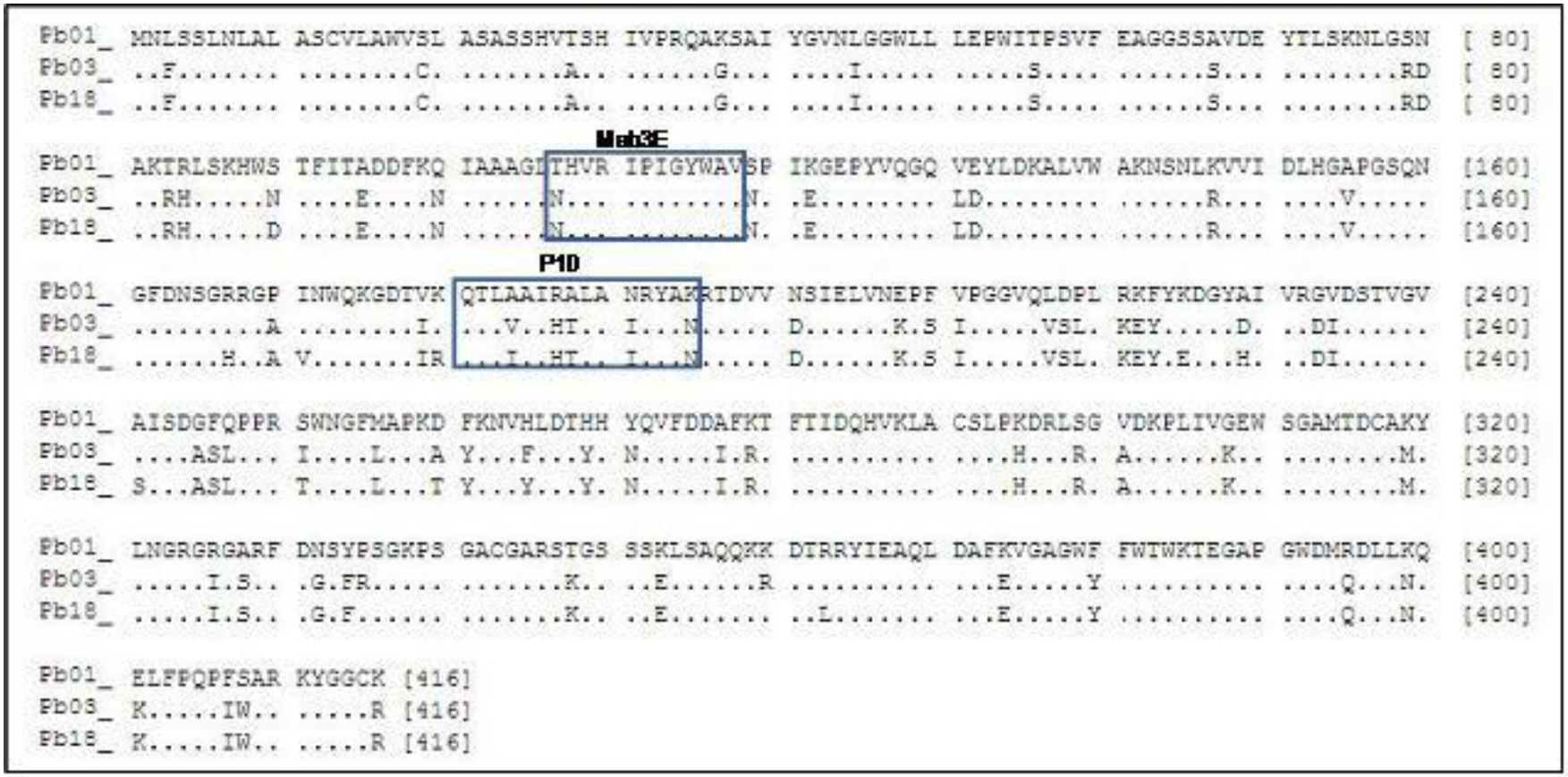
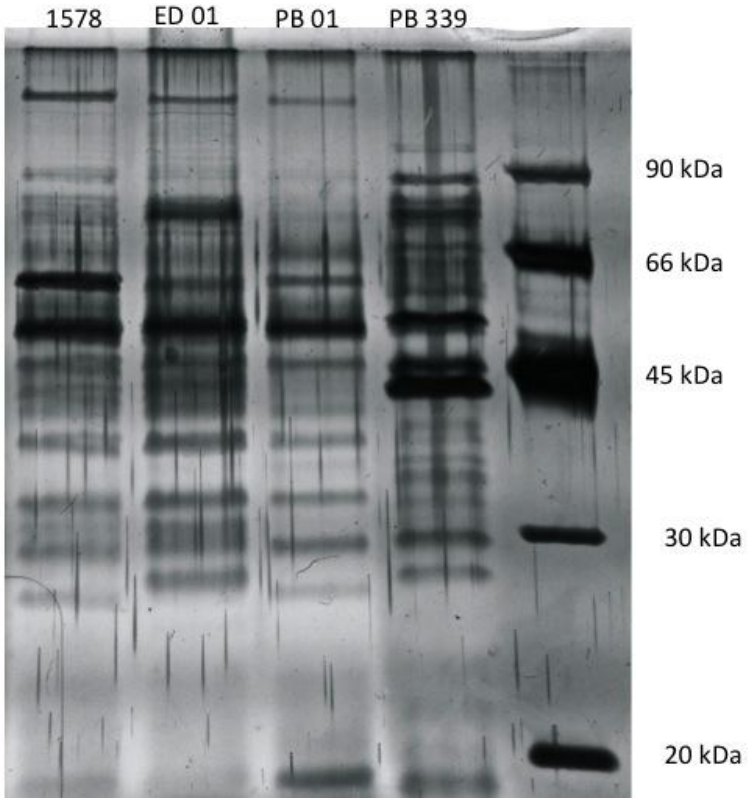


FIGURE 6 | Cytokine levels in the lungs obtained from BALB/c mice that received either 1 mg of polyclonal antibodies against acidic glycolipids or 1 mg anti- BSA polyclonal antibodies (control) 30 days after infection with Pb18 and sacrificed 45 and 60 days post-infection (therapeutic protocol). $^{*}p < 0.05$, $^{\#\#}p < 0.005$ (student's *t*-test).

Paracoccidioides lutzii



New strategies for vaccine development against *P. lutzii*

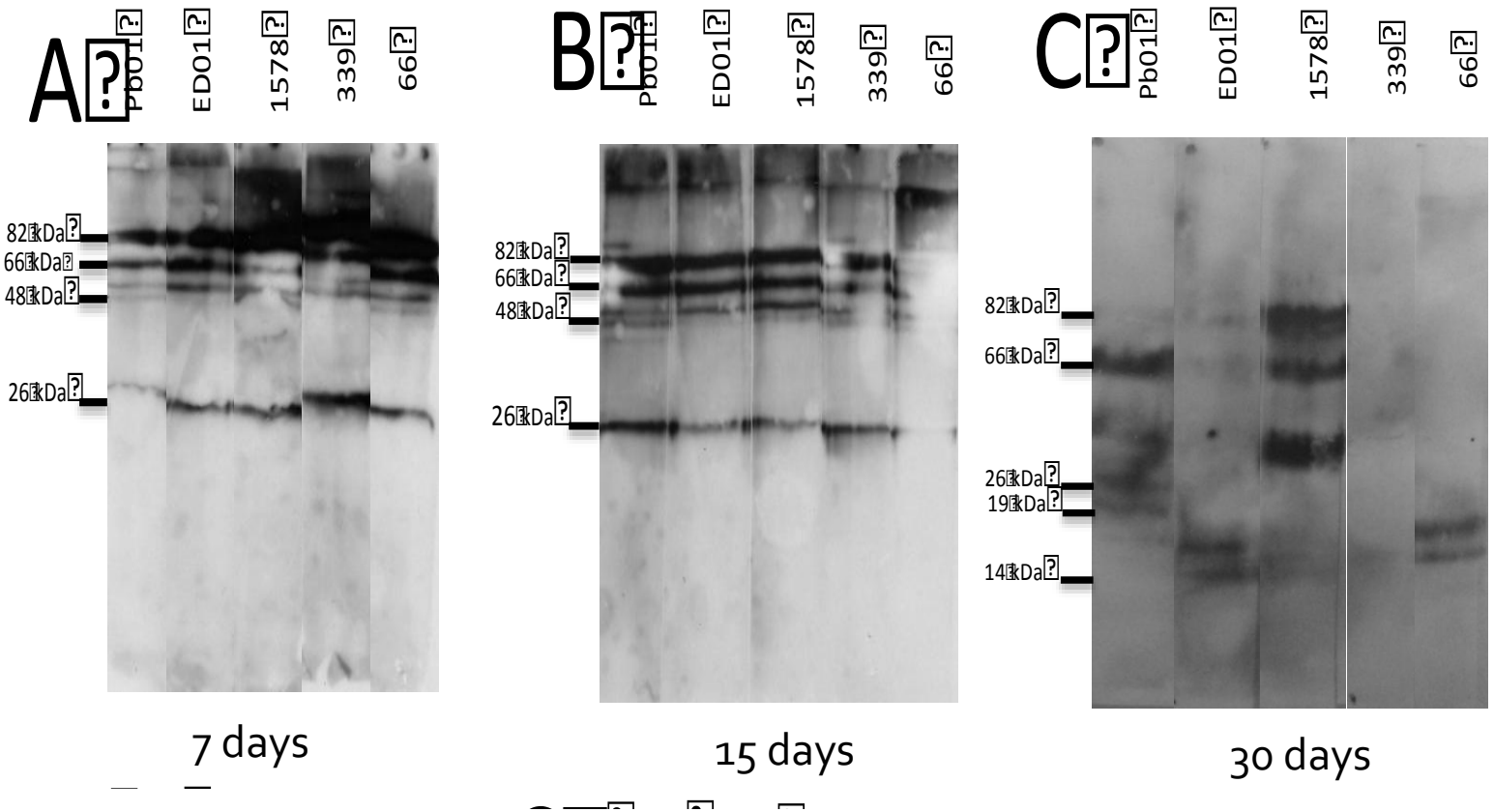


Electrophoresis of exoantigen from different isolates of *P. lutzii*

Diego Rossi – Ph.D. student

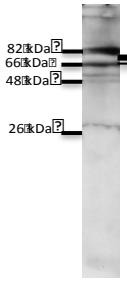
Western blot

serum from infected mice



1st strategy

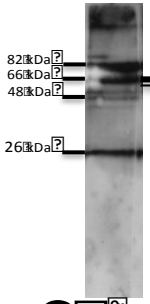
A



sequencing by Mass spectrometer

Uni Prot Code	Protein	Mass (KDa)
PADG_01945	PB18 GTP binding Sey1	98

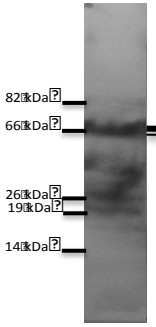
B



sequencing by Mass spectrometer

Uni Prot Code	Protein	Mass (Kda)
PADG_01752	PB18 conserved hypotetical protein	33

C



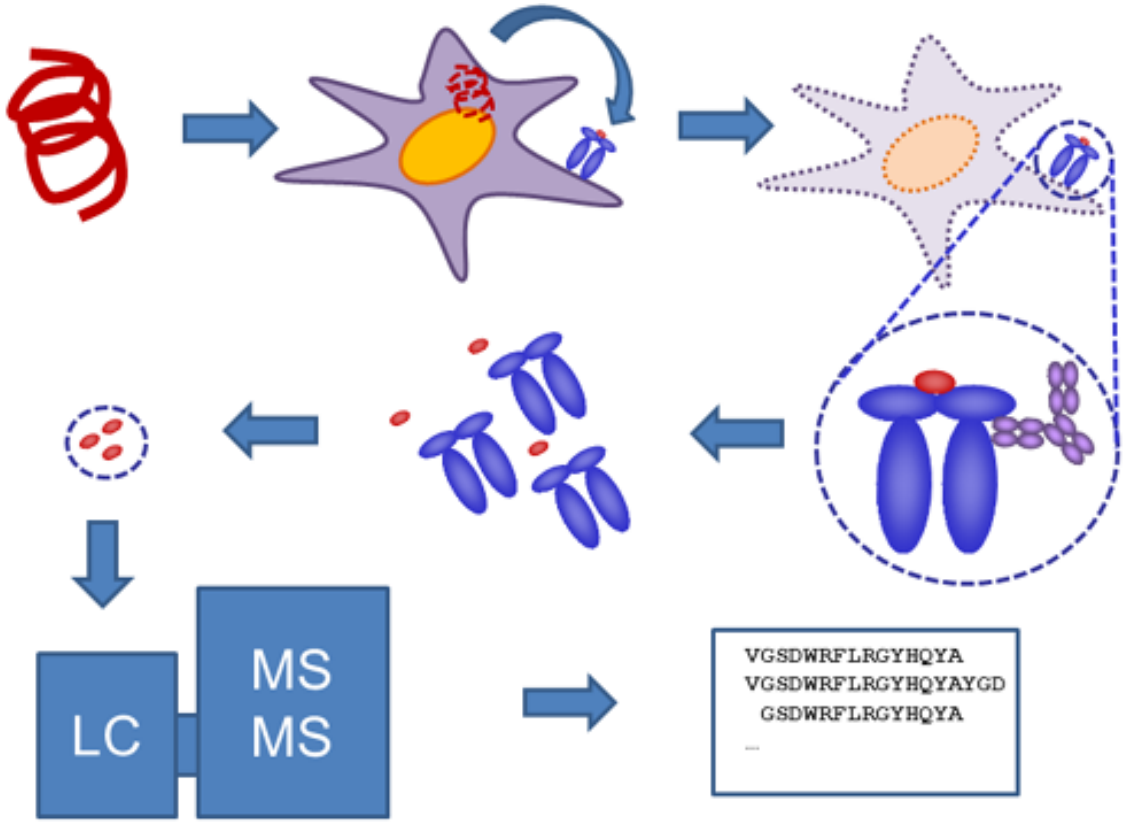
sequencing by Mass spectrometer

Uni Prot Code	Protein	Mass (Kda)
PABG_05309	PB03 SelenoCysteine Lyase	53
PABG_00384	PB03 Beta Chimaerin	124

Western Blot of antigen from cell wall extraction (A) 7 days, (B) 15 days and (C) exoantigen. PB01 was cultivated in Fava Netto's media at 37°C.
The reactive bands were sequenced by mass spectrometry and analysed by MASCOT using the *Paracoccidioides* genome bank.

Purification of peptides from MHC-II

2nd strategy



Johns Hopkins University
Arturo Casadevall

Paracoccidioides lutzii

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Original article

Monoclonal antibodies to heat shock protein 60 induce a protective immune response against experimental Paracoccidioides lutzii

Luciana Thomaz [a], Joshua D. Nosanchuk [b,c], Diego C.P. Rossi [a], Luiz R. Travassos [e], rlos P. Taborda [a,d,*]

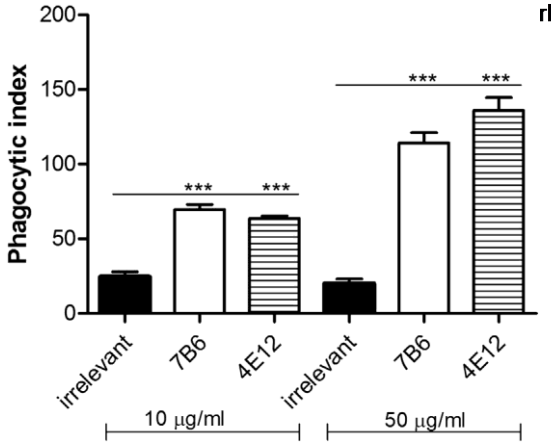

Fig. 2. **mAb to Hsp60 modify phagocytosis of *P. lutzii* by peritoneal macrophages**. Phagocytosis of *P. lutzii* by primary peritoneal macrophages from BALB/c mice after 24 h in the presence of two concentrations of mAbs against Hsp60 compared to phagocytosis of yeast cells with irrelevant mAb. Each experiment was done in triplicate. ***, $p < 0.0001$, comparing Hsp-binding mAb to control mAb.

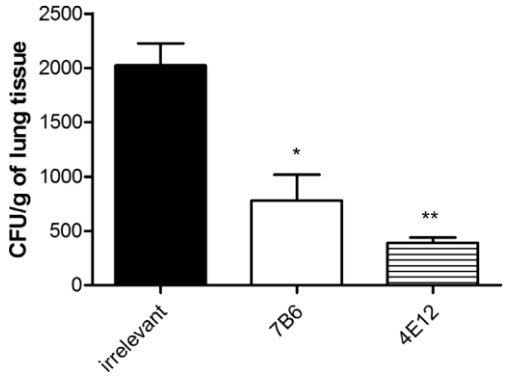

Fig. 3. **Hsp60-binding mAbs reduce *P. lutzii* fungal burdens in the lungs of infected mice**. Lung CFU from mice 15 days after infection with *P. lutzii*. The animals had received either Hsp60-binding mAb (7B6 or 4E12) or irrelevant mAb 24 h before IT infection with *P. lutzii* 01. Each bar represents the average of two similar experiments. *, $p < 0.05$ and **, $p < 0.005$, significant difference relative to the irrelevant control.

Paracoccidioides lutzii

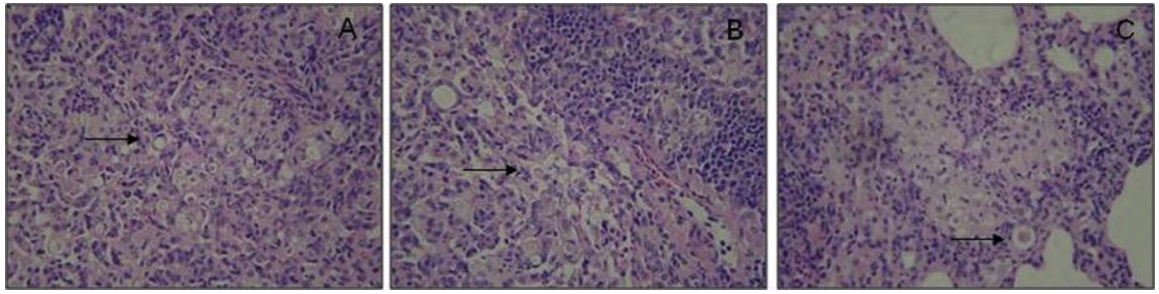

Fig. 5. **Hsp60-binding mAbs reduce the number of *P. lutzii* in the lungs of infected mice**. Hematoxylin and Eosin staining of lung sections from mice 15 days after infection with *P. lutzii*. The animals had received either Hsp60-binding MAb (7B6 or 4E12) or irrelevant mAb, 24 h before IT infection with *P. lutzii* 01. (A) irrelevant mAb, (B) mAb 7B6 and (C) mAb 4E12. × 40 magnification. Black arrows point to yeast cells.

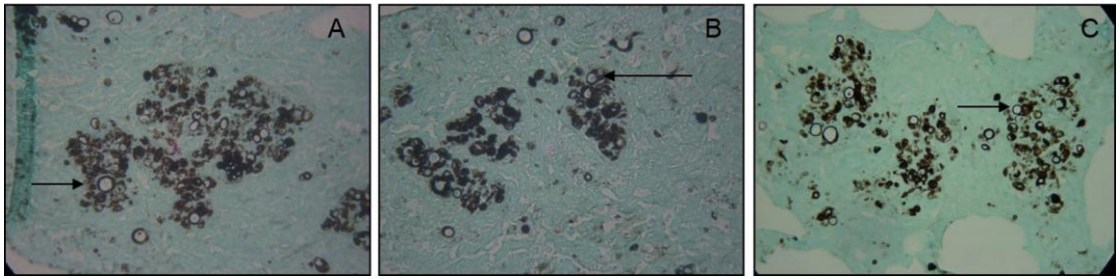

Fig. 4. **Gomoriś methenamine silver staining reveals that Hsp60-binding mAbs reduce the number of *P. lutzii* in the lungs of infected mice**. Gomoriś methenamine silver staining of lung sections from mice 15 days after infection with *P. lutzii*. The animals had received either Hsp60-binding MAb (7B6 or 4E12) or irrelevant mAb 24 h before IT infection with *P. lutzii* 01. (A) irrelevant mAb, (B) mAb 7B6 and (C) mAb 4E12. × 40 magnification. Black arrows point to yeast cells.

Laboratory

- **Universidade de São Paulo**
  - Carlos P. Taborda
    - Post-docs
      - Ana Camila Oliveira Souza
      - Julian E. Muñoz Henao
      - Jane Kaiano
      - Luciana Thomaz
      - Viviani Bressani
    - Alunos de Doutorado
      - Ágata N. D'Áuria Moura
      - Camila Boniche
      - Cleison Taira
      - Diego C. Rossi
      - Marcelo V. de Araújo
      - Martha E. Uran (Col)*
      - Leandro B. Roque
      - Lucas Dias
    - Alunos de mestrado
      - Elúzia C. P. Emidio*
      - Samuel Rodrigues
- **Universidade Federal de São Paulo**
  - Luiz R. Travassos
  - Zoilo Pires de Camargo
- **Albert Einstein College of Medicine**
  - Joshua D. Nosanchuk
- **Johns Hopkins University**
  - Arturo Casadevall
    - Apoio Financeiro
      - FAPESP
      - CNPq
      - CAPES