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PRESENCE OF C3 RECEPTORS ON DIFFERENT STRAINS OF *PARACOCCIDIOIDES BRASILIENSIS*.

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SUMMARY

Yeast forms of *Paracoccidioides brasiliensis* express C3 receptors, as determined by rosetting with erythrocytes carrying complement of C5 deficient mice. Rosetting with EAC was markedly reduced on pathogenic strains (Pb.18 and Pb.SN), and appears preferentially on non pathogenic strain (IVIC Pb.267) or on a strain of relatively low virulence (IVIC Pb.9). Rosetting with EAC was not reduced when yeast forms of all strains were heat killed.

Keywords: *Paracoccidioides brasiliensis*, immunology, experimental research.

INTRODUCTION

Human peripheral blood cells express several membrane glycoproteins that bind to cleavage products of the third component of complement (8,10,18,19). Receptors with these specificities are not found exclusively on mammalian cells, but also in microorganisms as demonstrated by HEIDENREICH and DIERICH (1985). The authors (9) showed that *Candida albicans* and *Candida stellatoidea* rosette with erythrocytes coated with iC3b (EAC3bi) and C3d (EAC3d). Later, the presence of *Candida* receptors for C3 fragments have been substantiated by EDWARDS et al., (7) and CALDERONE et al., (1). These investigators suggested that a lectin-like

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interaction occurred between these C3 fragments and *C. albicans*, since d-manose and d-glucose partially inhibited rosetting.

Several constituints of *Paracoccidioides brasiliensis* have been characterized (11,12,13,14,15) and correlated with virulence. Lipids and polisaccharides have been shown to be the most important of these constituints. In recent studies, SILVA (16) and SILVA & FAZIOLI (17) showed that these fractions are able to induce inflammatory response and consuption of complement by alternative and classical pathway.

In the present study we evaluated the ability of pathogenic and non pathogenic strains of *P. brasiliensis* to rosette with erythrocytes coated with complement of C5 deficient mice.

MATERIALS AND METHODS

Fungi: Four strains of *P. brasiliensis* were studied: Pb.SN and Pb.18, obtained from the culture collection of "Departamento de Microbiologia - Universidade de São Paulo" - Brazil; IVIC Pb.9 and IVIC Pb.267, isolated by Drs. Gioconda and Felipe San-Blas, and kindly supplied by Dra. Vera Calich (USP-Brazil). All these fungi were originally isolated from human patients, except IVIC Pb.267, a non pathogenic chemical mutant obtained after treatment of IVIC Pb.9 with nitrosoquanidine (14). IVIC Pb.9, a strain of relatively low virulence, led to the development of lesions in hamster, but it did not produce disease and could not be recovered from experimentally infected mice (13). *P. brasiliensis*, strain 18 is the most virulent (5,6).

Growth conditions: *P. brasiliensis* were cultivated in solid Fava Netto's medium (5,6); strains IVIC Pb.9 and IVIC Pb.267 were mantained in solid peptone-yeast extract glucose medium (13). All strains were cultivated in their yeast phase at 37°C for a week, with exception of IVIC Pb.267 which grows well at 23°C and has not a typical yeast-phase.

Preparation of E, EA and EAC: Sheep red blood cells 5% were optimaly sensitized with hemolisin for 15 minutes at 37°C, washed, incubated vol./vol. with serum of C5 deficient mice A/Sn) diluted 1:2. After incubation for 15 min. at 37°C,

the cells were washed and ressuspended to the initial volume. In all steps we used TBS containing Ca^{++} and Mg^{++} (3).

Rosetting assay: The fungi were washed in PBS, ressuspended in TBS with ions, and the viability analysed (2). Portions of E, EA and EAC 0.5% were incubated with equal volum of each fungi strain (0.5 ml) containing 10^6 viable L forms of *P. brasiliensis* for 30 minutes at 37°C. Samples were then removed, and the percentage of yeast cells with adhering erythrocytes was determined. Results were evaluated with light microscopy (magnification X 4000). At least 100 yeast forms were counted in each incubation mixture and adherence was defined as the binding of at least four erythrocytes to each yeast.

RESULTS

Rosetting with E, EA or EAC: In the table number 1, we show the percentage of interaction between the four strains of yeast with the complex E, EA, EAC. All yeast forms of *P. brasiliensis* rosette with EAC. The interaction of Pb.18 and Pb.SN (Pathogenic strains) was low and similar. Strain IVIC Pb.267 (non pathogenic strain) presents a good interaction with EAC, and strain IVIC Pb.9 was intermediary.

TABLE 1 - Rosetting of *P. brasiliensis* yeast cells of different strains with E, EA or EAC.

Complement intermediate	% yeast cell with rosettes[a]			
	IVIC Pb.267	IVIC Pb.9	Pb.SN	Pb.18
E	1.0 ± 0,7	0.9 ± 0.7	0.9 ± 0.5	1.0 ± 0.7
EA	3.0 ± 1.0	3.1 ± 1.1	3.2 ± 1.5	3.0 ± 2.0
EAC	31.0 ± 3.2	21.7 ± 1.8	11.2 ± 3.7	9.0 ± 3.9

a: percent rosetting was determined by couting 100 yeast particles. Data are expressed as the mean ± standart deviation for samples assayed in triplicate.

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Effects of heat on rosetting of EAC by yeast forms of *P. brasiliensis*: Rosetting of EAC wasn't abolished by heating of yeast forms of *P. brasiliensis* at 100°C for 1 h, for any strain tested. These results are showed in table 2.

TABLE 2 - Effect of previous treatment of *P. brasiliensis* with heat on EAC3 receptor activity.

Strain	% rosetting[a]	
	untreated	heat killed[b]
IVIC Pb.267	31.6 ± 1.7	32.7 ± 2.8
IVIC Pb.9	22.5 ± 1.8	20.3 ± 1.2
Pb.SN	9.8 ± 1.4	10.5 ± 2.1
Pb.18	11.0 ± 1.3	10.3 ± 2.4

a: data expressed as the mean ± standart deviation for samples assayed in triplicate
b: heat killing was done at 100°C for one hour.

DISCUSSION

The experiments carried out in the present study demonstrate that yeast forms of *P. brasiliensis* can interact with C3 fragments, and that this fenomen can be correlated with the virulence of the fungi. These receptors are heat resistant, since heat killed cells rosetting as well as viable cells.

Polysaccharides and lipids are considered to be one of the main fraction of the cell wall of *P. brasiliensis*. SILVA and FAZIOLI (17) observed an intense inflammatory response in the lungs of mice injected intravenously with these fractions, and demonstrated that rats injected intraperitoneally with this products showed a greater influx of PMN.

Influx of PMN cells to the site of inoculation of *P. brasiliensis* has been attributed to the ability of the fungus to activate the alternative complement pathway (3) and to the chemotactic activity of these cells induced by products of the complement activation (4).

The presence of a receptor for C3 fragments on *Candida sp* was described by HEIDENREICH and DIERICH (9). These receptors were observed on both, yeast and hyphal forms, whereas they were detected only on hyphal forms by EDWARDS et al., (7) and by CALDERONE et al., (1).

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Considerable emphasis has been placed on defining the biological consequences of the interactions of mammalian cell receptors with C3b and its futher cleavage products. The functional significance of the C3 receptors on *P. brasiliensis* remains unknown. The finding of more C3 receptors on non pathogenic strain of *P. brasiliensis* is suggestive of their envolvement in the disease processes. The mechanim by which these receptors may participate in the pathogenesis have not been defined, but C3 receptors may facilitate the adhesion of PMN and macrophages.

Considering that suggar may play a role in the interaction of fungi and C3b, futher investigation concerning the characterization of these receptors will be performed.

RESUMO

Presença de receptores C3 nas diferentes cepas de *Paracoccidioides brasiliensis*.

Formas leveduriformes de *Paracoccidioides brasiliensis* apresentaram receptores para C3, como determinado pela formação de rosetas com eritrócitos de carneiro sensibilizados com hemolisina e complemento de camundongos C5 deficientes. A formação de rosetas com EAC mostrou-se marcadamente reduzida para as cepas patogênicas (Pb.18 e PB.SN), e ocorreu preferencialmente quando se utilizou a cepa não patogênica (IVIC PB.267) ou uma cepa de virulência relativamente baixa (IVIC Pb.9). A formação de rosetas com EAC não foi reduzida quando as formas leveduriformes de todas as cepas analisadas foram submetidas a temperatura de 100°C.

Unitermos: *Paracoccidioides brasiliensis*, imunologia, trabalho experimental.

REFERENCES

01. CALDERONE, R. A.; LINEHHAN, L.; WADSWORTH, E. & SANDBERG, A.L. Identification of C3d receptors on *Candida albicans*. **Infect. Immun. 56**:252-258, 1988.

02. CALICH, V. L. G.; PURCHIO, A. & PAULA C. A. A new fluorescent viability test for fungi cells. **Mycopathologia 66**:175-177, 1978.

03. CALICH, V. L. G.; KIPNIS, T. L.; MARIANO, M.; FAVA NETTO, C. & DIAS DA SILVA, W. The activation of the complement system by *Paracoccidioides brasiliensis* in vitro. Its opsonic effect and possible significance for an in vivo model of infection. **Clin. Immunol. Immunopathol. 12**:20-30, 1979.

04. CALICH, V. L. G.; COPI VAZ, C. A. & BURGER, E. PMN chemotactic factor produced by glass-adherent cells in the acute fase of inflammation caused by *Paracoccidioides brasiliensis*. **Braz. J. Exp. Pathol. 66**:57-65, 1985.

05. CARVALHAES, M. S.; DIAS DA SILVA, W.; BIRMAN, E. G.; SANT'ANNA, O. A.; ABRAHAMSON, P. & KIPNIS, T. L. Experimental paracoccidioidomycosis in high and in low producers mice: II. The infection in F1 generation. **in**: Genetic Control of Host Resistance to Infection and Malignancy, Alan R. Liss, Inc., pg. 421-423, 1985.

06. CARVALHAES, M. S.; DIAS DA SILVA, W.; BIRMAN, E. G.; SANT'ANNA, O. A.; ABRAHAMSON. P. & KIPNIS, T. L. Experimental paracoccidioidomycosis in high and low antibody producers mice. I-Evolution of the disease, its correlation with the humoral immune response and the patterns of tissue lesions. **Ann. Inst.Pasteur/Immunol. 137C**:127-141, 1986.

07. EDWARDS, J. E.; GAITHER, T. A.; O'SHEA, J. J.; ROTRODEN, D.; LAWLEY, T. L.; WRIGHT, S. A.; FRANK, M. M. & GREEN, I. Expression of specific binding sites on *Candida* with functional antigenic characteristics of human complement receptors. **J. Immunol. 137**:3577-3583, 1986.

08. FRADE, R.; MYONES, B. L.; BAREL, M.; KRIKORIAN, O. & ROSS, G. D. Gp 140, a C3b-binding membrane component of lymphocytes, is the B cell C3dg/C3d receptor (CR2) and is distinct from neutrophil C3dg receptor (CR4). **Eur. J. Immunol. 15**:1192-1197, 1985.

09. HEIDENREICH, F. & DIERICH, M. P. *Candida albicans* and *Candida stellatoidea*, in contrast to other *Candida species*, bind iC3b and C3d, but not C3b. **Infect. Immun. 50**:598-600, 1985.

10. IIDA, K.; NADLLER, L. & NUSSENZWEIG, V. Identification of the receptor for the complement fragment C3d by mean of monoclonal antibody. **J. Exp. Med. 158**:1021-1033, 1983.

11. KANETSUNA, F.; CARBONELL, L. L.; MORENO, R. E. & RODRIGUES, J. Cell wall composition of the yeast and mycelial forms of *Paracoccidioides brasiliensis*. **J. Bacteriol. 97**:1030-1041 1969.

12. KANETSUNA, F. & CARBONELL, L. M. Cell wall glucans of the yeast and mycelial forms of *Paracoccidioides brasiliensis*. **J. Bacteriol. 101**:675-680, 1970.

13. MANOCHA, M. S.; SAN-BLAS, G. & CENTENO, S. Lipid composition of *Paracoccidioides brasiliensis*. Possible correlation with virulence of different strains. **J. Gen. Microbiol. 117**:147-154, 1977.

14. SAN-BLAS, G. & SAN-BLAS, F. Cell wall structure and virulence. A review. **Mycopathologia 62**:77-86, 1977.

15. SAN-BLAS, G. The cell wall of human pathogens: its possible role in host-parasite relationships. **Mycopathologia 67**:115-118, 1979.

16. SILVA, C. L. Granulomatous reaction induced by lipids isolated from *Paracoccidioides brasiliensis*. **Trans R. Soc. Trop. Med. Hyg. 79**:70-74, 1987.

17. SILVA, C. L. & FAZIOLI, R. A. A *Paracoccidioides brasiliensis* polysaccharide having granuloma-inducing, toxic and macrophage stimulanting activity. **J. Gen. Microbiol. 131**:1497-1501, 1985.

18. VIK, D. P. & FEARON, D. T. Neutrophils express a receptor for C3bi, C3dg and C3d that is distinct from CR1, CR2 and CR3. **J. Immunol. 134**:2571-2579, 1985.

19. WEIS, J. J.; TEDDER, T. F. & FEARON, D. T. Identification of a 145.000 Mr membrane protein as the C3d receptor (CR2) of human B lymphocytes. **Proc. Natl. Acad. Sci. USA 81**:881-885, 1984.