J Bras Patol Med Lab. 2022; 58: 1-7.

10.1900/JBPML.2022.58.400

Evaluation of the Lectin Pathway in the Serum of Patients with Chronic Chagas Disease by Detection of C4 by Elisa

Avaliação da via das lectinas no soro de pacientes com doença de Chagas crônica pela detecção de C4 por Elisa

Renan Polachini; Lorena Bavia; Fabiana A. Andrade; Kárita C. F. Lidani; Vanessa F. Picceli; Nathalia M. D. L. Signorini; Pâmela D. Fontana; Helena M. B. S. Plácido; Iara J. M. Reason

Universidade Federal do Parana, Department of Medical Pathology, Curitiba, PR Parana, Brazil.

ABSTRACT

Chagas disease (CD) is a chronic tropical disease caused by *Trypanosoma cruzi*, affecting about 8 million people in Latin America. The lectin pathway (LP) of the complement system is one of the first lines of host defense in the response against *T. cruzi*, and can continue to be activated in chronic infection due to the escape of the parasite to its action. Although some components of this pathway have been investigated in CD, there are no reports on its activation in patient serum. In this context, our objective was to evaluate the activation of LP in chronic chagasic patients and controls by the detection of the C4 component, using the direct ELISA assay. For this purpose, serum of 80 patient with chronic CD (clinical forms: asymptomatic n=17; symptomatic n=63; cardiac n=45; cardio digestive n=13; digestive n=5) followed at the Ambulatory of Attention to Chagasic Patients (HC/UFPR) and 80 healthy controls (donors of the Blood Bank of HC) were evaluated regarding the evaluation of the LP. The results showed that LP activation by mannose-binding lectin (MBL) was found reduced while activation by ficolins was increased in patients with CD when compared to controls. The same results were observed when the patients were categorized according to the indeterminate and symptomatic clinical forms. We conclude that the detection of the C4 component by ELISA is an efficient methodology to assess LP activation in serum from patients with chronic CD, enabling to differentiate the activation profile between patients and controls..

Key words: chagas disease; complement system; complement activation; lectin pathway; mannose-binding lectin; ficolins.

RESUMO

A doença de Chagas (DC) é uma doença tropical crônica causada pelo Trypanosoma cruzi, atingindo cerca de 8 milhões de pessoas na América Latina. A via das lectinas (VL) do sistema complemento é uma das primeiras linhas de defesa na resposta imunológica contra a infecção pelo T. cruzi, e pode continuar sendo ativada na infecção crônicadevido ao escape do parasito à sua ação. Embora alguns componentes dessa via tenham sido investigados na DC, não existem relatos sobre sua ativação em soro de pacientes. Neste contexto, nosso objetivo foi avaliar a ativação da VL no soro de pacientes com DC crônica e controles pela detecção do componente C4 empregando a técnica de ELISA. Para isso, amostras de soro de 80 pacientes com DC crônica (formas clínicas: indeterminada n=17; sintomática n=63; cardíaca n=45; cardiodigestiva n=13; digestiva n=5) atendidos no Ambulatório de Atenção ao Paciente Chagásico (HC/UFPR) e 80 controles saudáveis (doadores do Banco de Sangue do HC) foram avaliados quanto a ativação da VL. Os resultados demonstraram que a ativação da VL pela lectina ligante de manose (MBL) encontra-se reduzida, enquanto que a ativação pelas ficolinas está aumentada em pacientes com DC quando comparados aos controles. Os mesmos resultados foram observados quando os pacientes foram categorizados quanto às formas clínicas indeterminada e sintomática.

First submission on 03/10/2021; accepted for publication on 10/21/2021; published on 10/25/2021

Concluímos que a detecção do componente C4 por ELISA é uma metodologia eficiente para avaliar a ativação da VL em soro de pacientes com DC crônica possibilitando diferenciar o perfil de ativação entre pacientes e controles.
Unitermos: Strongyloides*; Covid-19; ciclo pulmonar; corticoides.*

INTRODUCTION

Chagas disease (CD) is considered a neglected disease, affecting about 8 million people in Latin America, according to the World Health Organization in 2019[1]. In Brazil, between 2007 and 2018, most cases of CD were registered. Brazilian states, with an annual average of 220 cases. In Brazil, the transmission of the protozoan Trypanosoma cruzi, which causes CD, has as one of the main vectors Triatoma infestans, an insect of the Triatominae subfamily. Regarding the probable forms of transmission occurring in the country, 73% were by oral transmission, 9% by vector transmission, 0.4% by vertical transmission, 0.1% by accidental transmission, and in 17.5% there was no identification of form of transmission, according to data from DATASUS[2,3].

The life cycle of T. cruzi consists of several evolutionary stages. After contamination of the host, the protozoan, which is initially found in the trypomastigotemetacyclic form, undergoes modifications to the reproductive amastigote form inside the host's cells. These transform into flagellate trypomastigotes, which are released after cell lysis and have the potential to infect other cells. During the acute phase, trypomastigotes migrate via the lymphatic and bloodstream to different tissues, mainly cardiac and smooth muscle, in addition to ganglia. Even after the development of a specific immune response against trypomastigotes, the parasite can persist in the host in the amastigote form in different tissues[4]. Although most individuals with CD remain asymptomatic, in the indeterminate form for life, 2-5% of them progress to each year, for one of the symptomatic forms of chronic CD: chronic Chagas cardiomyopathy (CCC), digestive megasyndromes, or both[5].

Immediately after inoculation of T. cruzi in the vertebrate host, the complement system (SC) is one of the main components of the innate defense acting to combat the parasite. This system consists of at least 35 plasma proteins and membrane receptors and can be activated in three pathways: classical (VC), lectins (VL) and alternative (VA). CV is typically initiated by immune complexes and requires the presence of the C1 (q,r,s) complex. VL is activated by the binding of pattern recognition molecules (PRMs), such as mannose-binding lectin (MBL) and ficolins, to pathogen-associated molecular patterns (PAMPs) and apoptotic cells (DAMPs); while VA is spontaneously activated by the hydrolysis of C3. All these pathways culminate in the cleavage of C3 and C5 components through convertases[6]. Once activated, several SC functions are generated, such as opsonization and phagocytosis of particles or microorganisms, potentiation of the inflammatory response by anaphylatoxins (C3a and C5a) derived from cleaved components, and cell lysis through the membrane attack complex (MAC), thus playing a fundamental role in the initial control of parasitemia by T. cruzi[7] Figure 1.

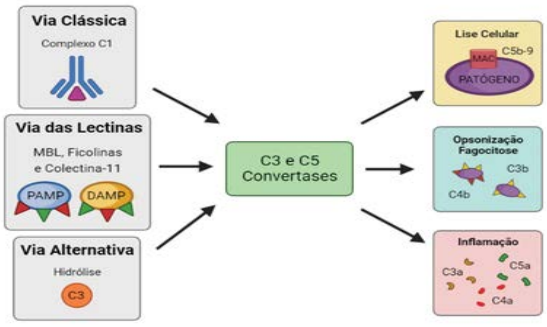

FIGURE 1 – *Complement system activation pathways*

VL is one of the first pathways to be activated in the presence of the parasite and plays an important role in the host's immune response against T. cruzi[8]. When MBL and ficolins bind to T. cruzi, the associated serine-protease enzymes to MBL (MASPs) are self-activated and cleave complement components C2 and C4, and their C2b and C4b products are deposited in the parasite, following the activation of the VL. This recognition and activation of the VL against T. cruzi occurs due to the presence of sugars on the surface of the parasite, such as mannose and n-acetylglucosamine, which promote the synergistic binding of MBL and ficolins to these compounds[9]. However, it is known that T. cruzi manages to evade SC activation and other defense mechanisms, establishing a chronic infection[7]. Considering the persistence of T. cruzi infection in chronically infected individuals, it is possible that an inadequate SC activation can promote tissue injury resulting from the inflammatory process, contributing to the clinical progression of the disease[10]. There are no reports in the literature on the functional assessment of VL activation in patients with chronic CD. The aim of the present study was to evaluate the systemic activation of VL by MBL and by ficolins in patients chronically infected with T. cruzi. In addition, with this work, we also propose a methodology to assess the activation of VL in the serum of chagasic patients.

METHODS

Study Design: This is an observational, analytical and cross-sectional study. The project was approved by the Research Ethics Committee of the Hospital de Clínicas of the Federal University of Paraná (HC-UFPR, Curitiba, Brazil) under number 15754513.70000.0096.

RECRUITMENT OF CONTROLS AND PATIENTS

Controls: The control group (n = 80) was composed of voluntary blood donors from the Biobanco Hospital de Clínicas da UFPR who had no clinical complaints[11] and had negative results for serology against T. cruzipelos by anti-T. cruzipor microparticle chemiluminescent immunoassay with sensitivity of 100% (95% CI: 97.90 to 100%) and specificity of 99.93% (95% CI: 99.80 to 99.99%) (Architect Plus Chagas, Abbott, USA), and indirect immunofluorescence with 100% sensitivity and specificity (IMUNO-Con Chagas, WAMA diagnostica, Brazil) [12,13]; In addition to negative results for other serological tests including: Treponema pallidum (flocculation test - VDRL), hepatitis B virus (anti-HBc and HBsAg, with IgG or IgG + IgM testing), hepatitis C virus (anti-HCV and HCV virus nucleic acid detection test, HIV (anti-HIV-1, anti-HIV-2, p24 antigen test and HIV virus nucleic acid detection test) and human T-celllymphotropic viruses 1 and 2 (anti-HTLV-1 and anti-HTLV-2).

Patients: The patients included in the study (n=80) were treated at the Chagas Patient Care Clinic at HC-UFPR. All patients included met the following characteristics: ≥18 years (no upper age limit), with clinical and serological diagnosis for CD, using the same tests used for controls. The following characteristics were used as exclusion criteria: pregnant women, lactating women, recent infections, suspected non-Chagas cardiomyopathy (such as hypertensive heart disease), unable to provide consent for the research, unable to answer the risk factor questionnaire due to disability or other factor, or who did not wish to participate in the study.

The clinical history and epidemiological data of the patients were obtained from medical records present in the medical records deposited in the archives of the Hospital de Clínicas, UFPR. In addition, an interview and general clinical examination were performed on all patients to determine other variables such as body mass index (BMI), blood pressure and diabetes. The collection of these data is intended to validate our study by comparing the epidemiology and comorbidities related to CD reported in the literature[14,15]. Patients were characterized as to the clinical form of CD based on clinical history, diagnosis and tests such as electrocardiogram of 12-lead, transthoracic echocardiogram, obtained from patients' charts[13].

Functional Assay of Lectin Pathway Activation by MBL and Ficolins: VL activation was evaluated in the serum of controls and patients with chronic CD, quantifying the presence of C4 molecules by enzyme-linked immunosorbent assay (ELISA), adapted from Bavia et al[16]. Polystyrene plates were sensitized with 100 µl/well of 10 µg/mL mannan solution (Sigma, USA) for the evaluation of VL activation by MBL or of 25 µg/mL acetylated bovine serum albumin (BSA-Ac, Promega, USA) for evaluation of VL activation by ficolins, in carbonate buffer (0.1 M NaHCO3, 0.1 M Na2CO3, pH 9.6) for 16 h at 4ºC. Plates were washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-T), blocked with 1% bovine serum albumin (BSA) in PBS for 60 min at 37°C. After washing, the plates were incubated for 60 min at 37°C with 100 µl/well of a pool of normal human serum (SHN) at different dilutions (for activation via MBL: 1:50, 1:67, 1:89, 1:118, 1:157, 1:209 and for activation via ficolins: 1:10, 1:20, 1:40, 1:80, 1:160, 1:320) in HEPES/NaCl++ buffer (10 mM HEPES) , 150 mMNaCl, 1 mM MgCl2, 2 mM CaCl2, 0.1% gelatin, pH 7.4) as standard curve. The SHN pool VL activity, used by default, gives the arbitrary value of one unit per mL (or 1000 mUA/mL). Thus, each SHN pool dilution corresponds to 1000, 750, 563, 422, 316, 237, 178 and 133 mUA/mL for activation via MBL, and 1000, 500, 250, 125, 62.5, 31.25 , 15.625 and 7.8125 mUA/ml for activation via ficolins. Sera from controls and patients were diluted 1:80 and 1:30 for VL activation assays by MBL and by ficolins, respectively, and incubated together with the respective standard curves. After washing, the plates were incubated for 60 min at 37°C with anti-human C4 antibody (ATB, USA) diluted 1:4000 in PBS-T containing 1% BSA (PTB). After incubation, the plates were washed again and incubated for 60 min at 37°C with the antibody conjugated with alkaline phosphatase anti-polyclonalanti-goat IgG (Sigma, USA) diluted 1:30000 in PTB. Development was performed after three washes, using 1 mg/mL of p-NitrophenylPhosphate substrate, Disodium Salt (PNPP, Sigma, USA) diluted in development buffer composed of 1 M diethanolamine supplemented with 0.5 mM MgCl2 in pH 9.8 and incubating the plate at 37°C. After 15 min of incubation, the first reading of the plates was carried out using an absorbance of 405 nanometers in the ELx800 spectrophotometer (Biotek, Brazil), and two more readings were taken every 15 min. Absorbance as a function of concentration is essentially non-linear, as the functional assay is characterized by a sequence of interdependent protein interactions. Considering the nature of the process, an exponential model was used to adjust the results of the standard curve in each test. The accuracy of the fit was evaluated

by the standard deviation of the fit or standard deviation of the fit in English (STDFIT), which is calculated as the root of the weighted sum of squares of the residuals divided by the number of degrees of freedom of the data. The STDFIT value ranged from 2.5% to 7.6% in relation to the mean absorbance value of the considered assays. The concentration of controls and patients was calculated by inverting the analytical expression, and the respective errors were estimated by the ratio between the STDFIT and the modulus of the derivative of the standard curve at the point. The final concentrations were obtained by the weighted average of the reading results, and the mean relative error of the calculated concentrations was 11%.

Statistical Analysis: The distribution of quantitative data was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov normality tests. Comparisons between quantitative data were performed using the non-parametric Mann-Whitney and Kruskal-Wallis tests. The results obtained were corrected by binary logistic regression adjusted for age, sex and ethnicity STATA v.9.2 (Statacorp, USA). For qualitative data, we used Fisher's exact test and Pearson's chi-square test. Correlation analysis was performed using Spearman's correlation test. P values less than 0.05 were considered significant (pAju-p adjusted or corrected). Data were presented as median and interquartiles. We employ the GraphPad Prism program – version 5.0.

RESULTS

Controls and Patients: The socio-demographic characteristics and comorbidities presented by the controls and patients are shown in Table 1. The mean age for the control group was 45 years, while for the patients it was 64 years. As for gender, the control group had 50% men and the patient group 40%. We observed a higher frequency of diabetics (12.5%) and hypertensive (75%) among patients, as well as a higher number of Afro-Brazilians affected by sores (21.2%), compared to controls (0.12 .5 and 7.5% respectively).

Assessment of Lectin Pathway Activation: The results of VL activation by MBL and ficolins in control subjects and chronic chagasic patients are shown in Figures 2A and 2B, respectively. Significant differences were observed between patients and controls for both evaluated pathways. There was a reduction by MBL and an increase by ficolins in the activity of VL and in patients with chronic CD when compared to controls.

Activation of the lectin pathway by MBL and by ficolins was evaluated in the presence of mannan (A) or acetylated BSA (B) by the ELISA technique. Data were presented as median and interquartile, n = 80 for each group. P values adjusted by binary logistic regression.

TABLE 1 – Socio-demographic characteristics and comorbidities in controls and patients

Characteristics	Controls n=80	Patients n=80	Indeterminaten=17	Cardiac n=45	Cardiodigestiven=13	Digestiven=5	Controls vs. Patients value of p
Age (years) median [máx. – min.]	45 [25 - 62]	64 [46 - 89]	64 [48 - 72]	63 [46 - 89]	66 [53 - 81]	61 [55 - 64]	p<0,0001
Sex, n (%)							
Women	40 (50,0%)	32 (40,0%)	8 (47,0%)	19 (42,2%)	5 (38.4%)	0 (0,0%)	p=0,2659
Mulheres	40 (50,0%)	48 (60,0%)	9 (53,0%)	26 (57,8%)	8 (61,6%)	5 (100%)	
Ancestry, n (%)							
Euro-Brazilian	72 (90,0%)	62 (77,5%)	14 (82,0%)	32 (71,1%)	11 (84,6%)	5 (100%)	p=0,0420
Afro-Brazilian	6 (7,5%)	17 (21,2%)	3 (18,0%)	12 (26,7%)	2 (15,4%)	0 (0,0%)	
Others	2 (2,5%)	1 (1,3%)	0 (0,0%)	1 (2,2%)	0 (0,0%)	0 (0,0%)	
Diabetes, n (%)	0 (0%)[*]	10 (12,5%)	0 (0%)	8 (17,8%)	1 (7,7%)	1 (20%)	p=0,0014
Hypertension, n (%)	10 (12,5%)[*]	61 (75%)	11(64,7%)	37 (82,2%)	9 (69,2%)	4 (80%)	p<0,0001
IMC (kg/m2), median	27,68[*]	28,42	28,06	28,44	26,3	32,2	p=0,0681
IMC Categorization[#] (kg/m^2), Median (n)							
Eutrophic	22,7	23,9	24,4	23,7	22,6	-	p=0,3372
Overweight	27,7	27,4	27,5	27,5	26,3	-	p=0,4013
Grade Obesity I	32,0	31,3	30,9	31,8	32,4	30,7	p=0,3557
Grade Obesity II	36,9	37,1	-	37,1	-	37,2	p=0,2810

NOTE: [*] The comorbidities assessed for controls were self-reported. [#] The body mass index (BMI) was classified according to the Brazilian Guidelines on Obesity, 2016 written by the Brazilian Association for the Study of Obesity and Metabolic Syndrome.

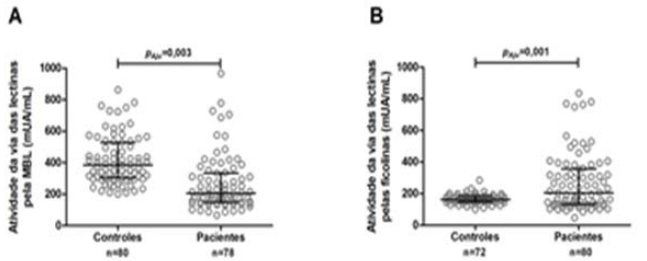

FIGURE 2 – *Functional assessment of lectin pathway activity in controls and patients with chronic Chagas disease*

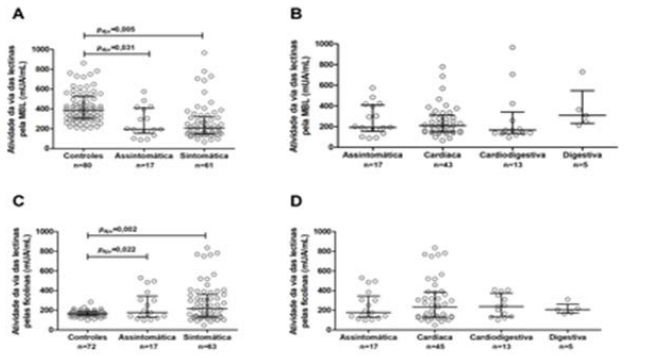

FIGURE 3 – *Functional assessment of lectin pathway activity in controls and in the different clinical forms of chronic CD*

Next, chronically infected patients were classified according to their clinical form and it was observed that the activation of VL by MBL was significantly lower in indeterminate and symptomatic patients, compared to controls Figure 3A. For VL activation by ficolins, a significant increase was observed among patients with the symptomatic and indeterminate clinical form when compared to controls Figure 3C. We found no significant difference between the cardiac, cardio digestive and digestive clinical forms for VL activation by MBL or by ficolins Figure 3B and D.

Activation of the lectin pathway by MBL and by phicholins was evaluated in the presence of mannan (A and B) or acetylated-BSA (C and D) by the ELISA technique. Data were presented as median and interquartile. P values adjusted by binary logistic regression.

There was no significant difference for any of the comorbidities in patients regarding VL activity: diabetes mellitus (MBL: pAju=0.407; ficolinaspAju=0.458), arterial hypertension (MBL: pAju=0.427; ficolinaspAju=0.176), and BMI categorized as eutrophicvs. Overweight and obese (grade I and II), (MBL: pAju=0.874; ficolins: pAju=0.929). No correlation was observed between VL activity and variables such as age, sex in patients or controls.

DISCUSSION

The results indicate that VL activation by MBL and ficolins have different response profiles in patients with chronic CD. Possibly, in the course of the infection, the differential activation of the VL may depend on factors arising from both the parasite and the tissue damage present in the chronic infection. This difference in VL activation by MBL and ficolins was also observed between patients with indeterminate and symptomatic form of CD compared to controls.

Although SC activation by T. cruzi is well known, as reviewed by Lidani et al[7], there are no reports on the detection of complement activation in patients with CD. However, some components of the complement system studied in isolation have been associated with chronic CD. VL is one of the first pathways to be activated in the presence of T. cruzi and the serum concentration of initiating components of this pathway has already been evaluated in chronic patients. It has been observed that low serum MBL levels are associated with protection against CCC[17], and serum MBL concentration increases with the severity of cardiac involvement[18] Corroborating these findings, a positive correlation between serum MBL and MBL complex levels /MASP-2/C4 was also previously observed[19]. In addition, altered levels of ficolin-2 were related to the degree of cardiac involvement[20]. Serum collectin-11 levels were significantly lower in patients with chronic CD compared to patients controls[21] So far, there are no reports on functional assays evaluating the three pathways of SC activation, nor on the products generated from the activation, in the serum of patients with chronic CD, nor on their impact on the natural history of the disease.

We observed a divergence in VL activation by MBL and by ficolins in patients, with a reduction in VL activation by MBL and an increase by ficolins compared to controls. It is possible that in the group of patients MBL is being recruited to act in processes arising from chronic infection such as the removal of apoptotic cells and cell debris, a function also attributed to MBL[22]. With this, the serum concentration of MBL could decrease due to to an increase in consumption, and consequently the activation of VL by MBL could be compromised, since the serum concentration of MBL is positively related to the concentrations obtained in the functional assay for VL[23]. On the other hand, caution must be exercised in the interpretation of the results regarding the activation of VL by ficolins, since in this assay acetylated BSA is used, a ligand recognized by the three ficolins (1 to 3)[24,25]. In this context, greater activity of VL by the ficolins was observed. Ficolins among patients with CD when compared to controls. It is

possible that the increased activation of VL by ficolins is related to a differential and synergistic response of ficolins[26-28], acting both in the inflammatory response in the chronic phase of the disease and in the removal of apoptotic cells and cellular debris[29-31].

Our study has some limitations. As this is a preliminary assessment, the number of patients used was low. However, the observed results may guide future studies, where a larger sample may confirm the change in VL activation in patients with chronic CD. Furthermore, in order to ensure greater confidence in the results obtained, all results were corrected by logistic regression, considering age as a covariate. Regarding the epidemiology and comorbidities of patients, our data are in accordance with the pattern shown in the literature where comorbidities such as hypertension, diabetes and obesity are frequently observed in patients infected with T. cruzi. Regarding ancestry, our data corroborate previous studies where there is a higher prevalence of CD among blacks and browns[14,15]. The method of functional assessment of VL activation was standardized by our group based on the following[32]. We have not tested samples whose values are known for VL activation by MBL and by ficolins due to the difficulty in acquiring commercial kits. However, this does not invalidate the results, since the differences observed between the group of patients with CD and the control group validate the measurement methodology. Therefore, although preliminary, the results present for the first time an evaluation of VL activation in patients with chronic CD, indicating an alteration in both MBL-mediated and ficolin-mediated activity. These hypotheses, however, need to be confirmed in future studies with a larger number of patients, performing the clinical staging of the symptomatic forms and quantifying the VL initiating proteins in the patients' serum.

CONCLUSION

Our results demonstrate the activation of VL in serum from patients with chronic CD, where activation of this pathway, by MBL and by ficolins, suggest different response performances between chagasic patients and controls. These results indicate a role for the VL of the complement system in the immunopathogenesis of chronic CD. In addition, the method employed was efficient to functionally assess the activation of VL in the serum of chagasic patients, as they are in agreement with data in the literature.

Acknowledgements: The authors thank CNPq, Fundação Araucária, PPSUS and CAPES for their financial support and scholarships. Lorena Bavia was a junior postdoctoral fellow by CNPq (420553 / 2017-7) and Renan Ribeiro Polachini was a scientific initiation fellow by PPSUS (agreement 045/2017 of the Araucária Foundation). We are also grateful to Professor Doctor Rafael Carvalho Barreto of the Federal Technological University of Paraná for fitting the results to the standard curve in functional assays for activating the lectin pathway by MBL and ficolins.

Conflict of Interest: All authors disclaim any conflict of interest.

REFERENCES

1. World Health Organization - WHO. Chagas Disease (American trypanosomiasis) Geneva: WHO; 2019.
2. Rassi A Jr, Rassi A, Marin-Neto JA. Chagas disease. Lancet. 2010; 375(9723): 1388-402.
3. SUS Informatics Department - DATASUS. Health, Epidemiological and Morbidity Information: database.
4. De Souza W, De Carvalho TM, Barrias ES. Review on Trypanosoma cruzi: Host Cell Interaction. Int J Cell Biol. 2010; 295394.
5. Cunha-Neto E, Chevillard C. Chagas disease cardiomyopathy: immunopathology and genetics. Mediators Inflamm. 2014; 683230.
6. Andrade FA, Lidani KCF, Catarino SJ, et al. Serine Proteases in the Lectin Pathway of the Complement System. Proteases Physiol Pathol. 2017; 10: 397-420.
7. Lidani KCF, Bavia L, Ambrosio AR, et al. The Complement System: A Prey of Trypanosoma cruzi. Front Microbiol. 2017; 8: 607.
8. Barnum SR. C4a: An Anaphylatoxin in Name Only. J Innate Immun. 2015; 7(4): 333-9.
9. Cestari Idos S, Krarup A, Sim RB, et al. Role of early lectin pathway activation in the complement-mediated killing of Trypanosoma cruzi. Mol Immunol. 2009; 47(3): 426-37.
10. Aiello VD, Reis MM, Benvenuti LA, et al. A possible role for complement in the pathogenesis of chronic chagasic cardiomyopathy. J Pathol. 2002; 197(2): 224-9.
11. National Health Surveillance Agency - ANVISA. Resolution of the collegiate board - RDC nº 34, 2014.
12. Flores-Chavez MD, Sambri V, Schottstedt V, et al. Evaluation of the Elecsys® Chagas Assay for the Detection of Trypanosoma cruzi- Specific Antibodies in a Multicenter Study in Europe and Latin America. J Clin Microbiol. 2018; 56(5): e01446-17.
13. Dias JCP, Ramos Jr. AN, Gontijo ED, et al. 2 nd Brazilian Consensus on Chagas Disease, 2015. Epidemiol. 2016; 49(1): 3-60.

14. Lidani KCF, Sandri TL, Castillo-Neyra R, et al. Clinical and epidemiological aspects of chronic Chagas disease from Southern Brazil. Rev Soc Bras Med Trop. 2020; 53: 1-10.
15. Martins-Melo FR, Alencar CH, Ramos AN Jr, et al. Epidemiology of mortality related to Chagas' disease in Brazil, 1999-2007. PLoS Negl Trop Dis. 2012; 6(2): e1508.
16. Bavia L, Dias Fontana P, Bovo F, et al. Inhibitory Effect of Supercritical Extracts from Arctium lappa L. on the Lectin Pathway of the Complement System. Chem Biodivers. 2019; 16(12): e1900401.
17. Luz PR, Miyazaki MI, Chiminacio Neto N, et al. Genetically Determined MBL Deficiency Is Associated with Protection against Chronic Cardiomyopathy in Chagas Disease. PLoS Negl Trop Dis. 2016; 10(1): e0004257.
18. Luz PR, Miyazaki MI, Neto NC, et al. High levels of mannose-binding lectin are associated with the risk of severe cardiomyopathy in chronic Chagas Disease. Int J Cardiol. 2010; 143(3): 448-50.
19. Boldt AB, Luz PR, Messias-Reason IJ. MASP2 haplotypes are associated with high risk of cardiomyopathy in chronic Chagas disease. Clin Immunol. 2011; 140(1): 63-70.
20. Luz PR, Boldt AB, Grisbach C, et al. Association of L-ficolin levels and FCN2 genotypes with chronic Chagas disease. PLoS One. 2013; 8(4): e60237.
21. Sandri TL, Andrade FA, Lidani KCF, et al. Human collectin-11 (COLEC11) and its synergic genetic interaction with MASP2 are associated with the pathophysiology of Chagas Disease. PLoS Negl Trop Dis. 2019; 13(4): e0007324.
22. Ogden CA, deCathelineau A, Hoffmann PR, et al. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. J Exp Med. 2001; 194(6): 781-95.
23. Petersen SV, Thiel S, Jensen L, et al. An assay for the mannan-binding lectin pathway of complement activation. J Immunol Methods. 2001; 257(2): 107-16.
24. Garred P, Genster N, Pilely K, et al. A Journey through the lectin pathway of complement-MBL and beyond. Immunol Rev. 2016; 274(1): 74-97.
25. Endo Y, Matsushita M, Fujita T. New insights into the role of ficolins in the lectin pathway of innate immunity. Int Rev Cell Mol Biol. 2015; 316: 49-110.
26. Bjarnadottir H, Arnardottir M, Ludviksson BR. Frequency and distribution of FCN2 and FCN3 functional variants among MBL2 genotypes. Immunogenetics. 2016; 68(5): 315-25.
27. Ishii M, Ohsawa I, Inoshita H, et al. Serum concentration of complement components of the lectin pathway in maintenance hemodialysis patients, and relatively higher levels of L-Ficolin and MASP-2 in Mannose-binding lectin deficiency. TherApher Dial. 2011; 15(5): 441-7.
28. Tizzot MR, Lidani KCF, Andrade FA, et al. Ficolin-1 and Ficolin-3 Plasma Levels Are Altered in HIV and HIV/HCV Coinfected Patients From Southern Brazil. Front Immunol. 2018; 9: 2292.
29. Ma YJ, Doni A, Romani L, et al. Ficolin-1-PTX3 complex formation promotes clearance of altered self-cells and modulates IL-8 production. J Immunol. 2013; 191(3): 1324-33.
30. Jensen ML, Honoré C, Hummelshøj T, et al. Ficolin-2 recognizes DNA and participates in the clearance of dying host cells. Mol Immunol. 2007; 44(5): 856-65.
31. Honoré C, Hummelshoj T, Hansen BE, et al. The innate immune component ficolin 3 (Hakata antigen) mediates the clearance of late apoptotic cells. Arthritis Rheum. 2007; 56(5): 1598-607.
32. Kjaer TR, Thiel S. Assay for estimation of the functional activity of the mannan-binding lectin pathway of the complement system. Methods Mol Biol. 2014; 1100: 131-9.

CORRESPONDING AUTHOR

Renan Polachini 0000-0002-1219-8750
renanpolachini@gmail.com