DR CECILIA PARODI (Orcid ID : 0000-0001-5589-1443)

Article type : Brief Definitive Report

Title

B cell profile, B-cell activating factor concentration and IgG levels in human cutaneous and mucosal leishmaniasis

Running title:

B-cell alterations in human leishmaniasis

Author names and affiliations

Cecilia Parodi[1], Alejandra Barrio[2], María F. García Bustos[1], Ana G. González Prieto[2], Julia Pimentel[1], Noel Badano[3], María C. Albareda[4], Melisa E. Castro Eiro[4], Susana A. Laucella[4], and María M. de Elizalde de Bracco[3]

[1]Instituto de Patología Experimental-CONICET, Universidad Nacional de Salta, Salta, Argentina, [2]Cátedra de Microbiología, Facultad de Ciencias de la Salud, Universidad Nacional de Salta, Salta, Argentina, [3]Laboratorio de Inmunología, Instituto de Medicina Experimental-CONICET, Academia Nacional de Medicina, Buenos Aires, Argentina. [4]Instituto Nacional de Parasitología Dr. M. Fatala Chaben, Buenos Aires, Argentina

Corresponding author

Cecilia Parodi; *e-mail*: ceciliaparodi@conicet.gov.ar
Phone and fax number: 54-387-4255333
Postal address: Cecilia Parodi, Instituto de Patología Experimental-CONICET, Universidad Nacional de Salta, Av. Bolivia 5150, CP4400, Salta, Argentina.

Conflict of interest: The authors declare that they have no conflict of interest.

Data availability: The data that supports the findings of this study are available from the corresponding author upon reasonable request.

Author contributions: C.P. designed and performed the experiments and wrote the manuscript; A.B. obtained samples of lesions, made the diagnosis of leishmaniasis and performed the identification of *Leishmania* species; M.F.G.B. obtained samples of lesions and conducted clinical follow-up of patients; A.G.G.P. performed cultures of parasites and identification of *Leishmania* species; J.P., N.B., M.C.A. and M.E.C.E. performed ELISA and CBA analysis; S.A.L. and M.M.E.B. participated in the design, discussion and edition of the final version of the manuscript.

Abstract

Aims:. The aim of this study was to evaluate characteristics of B cells in human tegumentary leismaniasis (TL) analyzing cutaneous leishmaniasis (CL), most prevalent form and mucosal leishmaniasis (ML), aggressive form characterized by the destruction of the oral-nasal-pharyngeal cavities.

Methods and results: By flow cytometry analysis we found decreased percentages of nonclass-switched memory B cells in TL with the degree of the loss related to clinical severity. Using commercial ELISA, we reported high levels of B-cell activating factor (BAFF) and IgG preferentially in aggressive CL and markedly in ML together with decreased BAFF receptors in the latter. We also found lower levels of BAFF after clinical recovery suggesting a relation between BAFF and disease activity.

ML history of therapeutic failure presented high levels of BAFF accompanied by detectable concentrations of IFN-γ and IL-6 (assayed by commercial ELISA and cytometric bead arrays respectively), cytokines involved in exaggerated inflammatory responses and tissue damage in TL.

Conclusion: We demonstrate B cell disturbances in TL with the degree of the alterations related to clinical severity. We suggest a relation between excess of BAFF and disease activity and point towards a possible implication of BAFF in the inflammatory phenomenon of ML.

Keywords

B-cell activating factor; Cutaneous leishmaniasis; Mucocutaneous leishmaniasis; B lymphocytes; Hypergammaglobulinemia; Interferon-gamma; Interleukin-6

1. Introduction

B cells have have the ability to produce antibodies but are also involved in antigen presentation, cytokine production and activation of T cells [1]. Most reports analyzing tegumentary *Leishmania* strains suggest dual roles for B cells, contributing to disease susceptibility or enhancing protection [2], [3], depending on the experimental model and the parasite strain used. Although trypanosomatids are able to hinder and skew B cell functions to favor their own survival [1], the role of the humoral response in human tegumentary leishmaniasis (TL) is currently still scant. The peripheral memory B cells can be characterized by their surface expression of IgD and CD27 [4]. B cell responses are also guided byB cell activating factor (BAFF), crucial regulator ofperipheral B cell homeostasis[5], [6]. whose overproduction leads to detrimental effects in autoimmune disorders[7], [8]. Less is known about BAFF in microbial diseases, withelevated levels associated with disease progression, expansion of atypical memory B cells and inefficient antibody response in plasmodium and HIV infections [9], [10], [11], [12]. Increased BAFF has also been reported in visceral leishmaniasis [13] but its participation in TL remains unknown.

To describe the characteristics of B cells in TL, we analyzed plasma BAFF and total IgG, BAFF receptors, B cell phenotype and the presence of autoantibodies in cutaneous leishmaniasis (CL),the most prevalent form, in comparison to mucosal leishmaniasis (ML), chronic and aggressive form characterized by the destruction of the oral-nasal-pharyngeal cavities [14], [15]. Our aim was to determine if B cell disturbances are frequent in TL and if the degree of the alterations is related to distinct clinical forms.

2. Material and methods

2.1. *Study groups*

The study population comprisedpatients from the Northwest of Argentina diagnosed by clinical evaluation, identification of amastigotes,culture of parasites from lesion samples and detection of *Leishmania* DNA by using k-DNA-based PCR[16].

Table 1 shows the clinical characteristics of the patients and the control group (HS). The analytical approaches were performed irrespectively of the condition for anti- *T. cruzi* antibodies after verifying that there were not significant differences between CL vs. *T. cruzi*-seropositive CL and ML vs. *T. cruzi*-seropositive ML groups.

2.2. *Leishmania spp. identification*

DNA extraction (from samples obtained from lesions and culture-isolated parasites)and polymorphism Specific–PCR were performed as previously described[17].

2.4. *Blood sampling procedure*

Plasma sample separation and isolation of peripheral blood mononuclear cells were performed as previously described [18].

2.5. *Measurement of anti-T. cruzi antibodies*

Testing for anti-*T. cruzi* antibodies was carried out using a commercial kit (recombinant ELISA *v.3.0*, Wiener, Argentina).

2.6. *B cell phenotype*

FITC, PE or PerCP labeled anti-CD19, anti-CD27, anti-IgD, anti-BAFF-R, and anti-TACI monoclonal antibodies (Becton Dickinson, San José, CA, USA) were used. The cells were evaluated using a FACScan flow cytometer and FCS 3 Express, De Novo Software (Los Angeles, CA, USA).

2.7. *ELISA and cytometric bead arrays (CBA)*

The plasma concentrations of BAFF (Adipogen, San Diego, USA), total IgG (Bethyl, Texas, USA), IgG dsDNA antibodies (IMTEC, Wiesbaden, Germany) and IFN-γ (BD Biosciences, San Diego, USA) were determined by commercial ELISA. IL-6 was measured by CBA (BD

Biosciences, San Diego, USA) using a FACScalibur flow cytometer and the BD CBA Software, version 1.4.

2.8. *Statistical analysis*

A level of $p < 0.05$ was accepted as being statistically significant. The normality of the variable distribution was assessed by the D'Agostino & Pearson omnibus test. Continuous variables between two groups were compared with the Mann-Whitney U-test A nonparametric Kruskal-Wallis test with Dunn's post-test was used to compare differences among three or more groups. The data were analyzed with GraphPad Prism 6 (GraphPad Software, San Diego, CA).

3. Results

3.1. *Identification of the causative agent of CL and ML*

Leishmania spp. could be identified in 23 CL and in 11 ML samples (Table 1). In the rest of the samples, we obtained negative PS-PCR results after DNA extraction due to a low number of parasites in the chronic lesions.*L. (V.) braziliensis* was found in the majority of the CL and ML cases, followed by *L. (L). amazonensis*.

3.2. *Peripheral B cell subsets in TL*

Gating on $CD19^+$ B cells, we analyzed naïve (IgD^+CD27^-); nonclass-switched (IgD^+CD27^+), class-switched (IgD^-CD27^+) and double-negative (IgD^-CD27^-) B cells. We compared CL with single lesions (CL-S); CL with multiple lesions (CL-M) and ML. Both CL groups and more markedly ML patients showed significantly lower percentages of nonclass-switched memory B cells in comparison with the HS group (Fig. 1B). The gating strategy is shown in Figure 2.

3.3. *Plasma concentrations of BAFF and total IgG in active TL*

We next evaluated crucial factors and found elevated levels of BAFF and total IgG in CL-M and in ML (Fig. 3A and 3B) compared with the HS group. When patients were split into those with active lesions and those who showed complete clinical recovery (Table 1)BAFF levels were significantly lower in the groups with clinical improvement in contrast to CL-M and more markedly ML (Fig. 3A).

3.4. *BAFF levels and proinflammatory cytokines in ML*

We assessed proinflammatory cytokines in ML because IFN-γ contributes to tissue damage and IL-6 to active disease in aggressive forms of leishmaniasis [19]. We compared the concentration of BAFF in 12 ML patients with detectable IFN-γ [39.75 (20-112.8) pg/ ml; median (range)] and in 8 ML patients with undetectable IFN-γ (Figure 4A). Besides, we compared the levels of BAFF in 11 ML patients with IL-6 median concentration of 5.32 (1.25–33.91) pg/ ml and in 5 ML patients with absence of IL-6 (Figure 4B). We found higher levels of BAFF within the groups with detectable cytokines. Although aggressive nasopharyngeal lesions characterize the entire ML cohort, the majority of the ML patients with history of therapeutic failure (Table 1) showed simultaneous detection of BAFF, IFN-γ and IL-6 (Figure 4).

3.5. *Expression of the BAFF receptors in patients with ML*

We measured the expression of BAFF-receptor (BAFF-R) andTACI on $CD27^{+}CD19^{+}$ memory B cells from the CL-S, CL-M and ML groups.

Both receptors were diminished only in ML compared with the HS group (Fig. 5A and 5B).

3.6. Detection of *anti-double-stranded DNA antibodies (dsDNA antibodies) in TL*

To evaluate the production of autoantibodies, we assessed dsDNA antibodies, markers of Systemic lupus erythematosus (SLE)also detected in infections as chronic hepatitis C [20]. We assayed 22 CL and 22 ML samples, with the absence of these antibodies in all but one of the tested samples (Table 2).

4. Discussion

In the present work we found that*L. (V.) braziliensis* is the predominant causative agent of CL and ML in the Northwest of Argentina according to previous data from the group [21].

. We report that nonclass-switched memory B cells, essential in secondary immune responses [4], are decreased in CL and markedly diminished in ML, suggesting a relation between the degree of the loss and clinical severity. This subset was also diminished in malaria [22], rheumatoid arthritis [23] and HIV infections [24].

. We then analyzed BAFF, a member of the trimeric TNF family crucial for B cell function [25]. We report that in TL high levels of BAFF and of total IgG are more frequently found in aggressive clinical forms, named CL with multiple lesions and ML. BAFF demonstrated an impact over the nonclass-switched memory B subset [26], so it could be affecting the memory B cell composition in TL. In addition, only ML showed diminished BAFF receptors on memory B cells, in agreement with autoimmune diseases whereas the lower expression of BAFF receptors was associated with more severe disease [27], [28]. In contrast, recovered CL and ML patients showed lower BAFF levels than their active disease counterparts. These observations suggest that in TL an excess of BAFF might be related to disease activity, as previously reported for autoimmune diseases [27] and malaria [29]. The induction of BAFF could be favored by antigen persistence, as other protozoan antigens have shown the ability to induce this molecule in experimental conditions [30]. An exaggerated inflammatory response directed by IFN-γ is mainly responsible for the tissue damage in TL [31,32] and although less is known about IL-6 [33], increased levels were found in active visceral leishmaniasis [19]. We report that in the majority of ML cases with history of therapeutic failure BAFF was accompanied by the detection of IFN-γ and IL-6. These findings suggest that BAFF could contribute to the inflammatory phenomenon produced in ML.

The absence of antibodies to DNAused as unique markers of autoimmunity [34] in almost all of the studied patients suggest that in TL, the activation of B cells might not necessarily be linked to autoantibodies production, although assessment of a wider set of antibodies is needed to validate this idea.

Our work demonstrates that the degree of the B cell alterations is related to clinical severity in TL involving changes in peripheral B cell subsets, BAFF and IgG levels and expression of BAFF receptors. We suggest a relation between BAFF and disease activity in TL and indicate a possible implication of this molecule in the inflammatory phenomenon of ML.

Acknowledgements

The authors would like to thank the Dermatology Service of Hospital Señor del Milagro and the Dermatology/Otorhinolaryngology Services of Hospital San Bernardo, Salta, Argentina. We also would like to thank Dr. María C. Mora and Prof. Federico Ramos for their technical support and Dr. Patricia Baré for carefully reading of the entire manuscript.

Funding: This work was supported by a grant from the Alberto J. Roemmers Foundation, Argentina.

Ethical Approval: All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individuals participants included in the study.

References

1. Silva-Barrios S, Charpentier T, Stäger S. The Deadly Dance of B Cells with Trypanosomatids. Trends Parasitol 2018;34(2):155-171. doi:10.1016/j.pt.2017.10.001
2. Gibson-Corley KN, Boggiatto PM, Bockenstedt MM, Petersen CA, Waldschmidt TJ, Jones DE. Promotion of a Functional B Cell Germinal Center Response after Leishmania Species Co-Infection Is Associated with Lesion Resolution. AJPA 2012;180(5):2009-2017. doi:10.1016/j.ajpath.2012.01.012
3. Wanasen N, Xin L, Soong L. Pathogenic role of B cells and antibodies in murine Leishmania amazonensis infection. Int J Parasitol 2008;38:417-429. doi:10.1016/j.ijpara.2007.08.010
4. Shi Y, Agematsu K, Ochs HD, Sugane K. Functional analysis of human memory B-cell subpopulations: IgD+CD27+ B cells are crucial in secondary immune response by producing high affinity IgM. Clin Immunol 2003;108(2):128-37.5. Kiyama K, Kawabata D, Hosono Y, et al. Serum BAFF and APRIL levels in patients with IgG4-related disease and their clinical significance. Arthritis Res Ther 2012;14(2):1-8. doi:10.1186/ar3810
6. Mackay F, Sierro F, Grey ST, Gordon TP. Cracking the BAFF code. Nat Rev Immunol 2009;9(7):491-502. doi:10.1038/nri2572.
7. Mackay F, Sierro F, Grey ST, Gordon TP. The BAFF/APRIL system: an important player in systemic rheumatic diseases. Curr Dir Autoimmun 2005;8:243–65.
8. Tangye SG, Bryant VL, Cuss AK, Good KL. BAFF, APRIL and human B cell disorders. Semin Immunol 2006;18(5):305-317.doi:10.1016/j.smim.2006.04.004
9. Fontaine J, Chagnon-Choquet J, Valcke HS, Poudrier J, Roger M. High expression levels of B lymphocyte stimulator (BLyS) by dendritic cells correlate with HIV-related B-cell disease progression in humans. Blood 2011;117(1):145-155. doi:10.1182/blood-2010-08-

301887

10. Scholzen A, Teirlinck AC, Bijker EM, et al. BAFF and BAFF Receptor Levels Correlate with B Cell Subset Activation and Redistribution in Controlled Human Malaria Infection. J Immunol 2014;192(8):3719-3729. doi:10.4049/jimmunol.1302960
11. Portugal S, Tipton CM, Sohn H, et al. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. Elife 2015;1-21. doi:10.7554/eLife.07218
12. Borhis G, Trovato M, Chaoul N, Ibrahim HM, Richard Y. B-cell-activating factor and the B-cell compartment in HIV/SIV infection. Front Immunol 2017;8:1338. doi:10.3389/fimmu.2017.01338
13. Goto Y, Omachi S, Sanjoba C, Matsumoto Y. Short report: Elevation of serum B-cell activating factor levels during visceral leishmaniasis. Am J Trop Med Hyg 2014;91(5):912-914. doi:10.4269/ajtmh.14-0260
14. David CV, Craft N. Cutaneous and mucocutaneous leishmaniasis. Dermatol Ther 2009;22(6):491-502. doi:10.1111/j.1529-8019.2009.01272.x
15. Goto H, Lindoso JA. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. Expert Rev Anti Infect Ther 2010;8(4):419-433. doi:10.1586/eri.10.19
16. Barrio A, Mora MC, Ramos F, Moreno S, Samson R, Basombrío MA. Short report: Use of kDNA-based polymerase chain reaction as a sensitive and differentially diagnostic method of American Tegumentary Leishmaniasis in disease-endemic areas of northern Argentina. Am J Trop Med Hyg 2007;77(4):636-639. doi:10.1053/joca.2002.0851
17. Barrio A, García Bustos MF, Mora MC, Parodi C, Ramos F, Moreno S. Identificación por PS-PCR de especies de Leishmania y su correlación con características clínicas, epidemiológicas y terapéuticas en Salta, Argentina. Rev Argentina Salud Pública 2009;1(1):30-33.
18. Parodi C, García Bustos MF, Barrio A, et al. American tegumentary leishmaniasis: T-cell differentiation profile of cutaneous and mucosal forms—co-infection with Trypanosoma cruzi. Med Microbiol Immunol 2016;205(4):353-369. doi:10.1007/s00430-016-0455-0
19. Van der Poll T, Zijlstra EE, Mevissen M. Interleukin 6 during active visceral leishmaniasis and after treatment. Clin Immunol Immunopathol 1995;77(1):111-114.
20. Palazzi C, Buskila D, Angelo SD, Amico ED, Olivieri I. Autoimmunity Reviews

Autoantibodies in patients with chronic hepatitis C virus infection : pitfalls for the diagnosis of rheumatic diseases. Autoimmun Rev 2012;11(9):659-663. doi:10.1016/j.autrev.2011.11.011

21. García Bustos MF, González Prieto G, Ramos F, et al. Clinical and epidemiological features of leishmaniasis in northwestern-Argentina through a retrospective analysis of recent cases. Acta Trop 2016;154:125-132. doi:10.1016/j.actatropica.2015.11.008
22. Asito AS, Piriou E, Jura WG, et al. Suppression of circulating IgD+CD27+ memory B cells in infants living in a malaria-endemic region of Kenya. Malar J 2011;10(1):362. doi:10.1186/1475-2875-10-362
23. Moura RA, Weinmann P, Pereira PA, et al. Alterations on peripheral blood B-cell subpopulations in very early arthritis patients. Rheumatology 2010;49(6):1082-1092. doi:10.1093/rheumatology/keq029
24. Jacobsen MC, Thiébaut R, Fisher C, et al. Pediatric Human Immunodeficiency Virus Infection and Circulating IgD $^{+}$ Memory B Cells. J Infect Dis 2008;198(4):481-485. doi:10.1086/590215
25. Mackay F, Schneider P, Rennert P, Browning J. BAFF and APRIL: A Tutorial on B Cell Survival. Annu Rev Immunol 2003;21(1):231-264. doi:10.1146/annurev.immunol.21.120601.141152
26. Scholz JL, Crowley JE, Tomayko MM, et al. BLyS inhibition eliminates primary B cells but leaves natural and acquired humoral immunity intact. Proc Natl Acad Sci 2008;105(40):15517-15522. doi:10.1073/pnas.0807841105
27. Salazar-Camarena DC, Ortiz-Lazareno PC, Cruz A, et al. Association of BAFF, APRIL serum levels, BAFF-R, TACI and BCMA expression on peripheral B-cell subsets with clinical manifestations in systemic lupus erythematosus. Lupus 2016;25(6):582-592. doi:10.1177/0961203315608254
28. Sellam J, Miceli-Richard C, Gottenberg JE, et al. Decreased B cell activating factor receptor expression on peripheral lymphocytes associated with increased disease activity in primary Sjögren's syndrome and systemic lupus erythematosus. Ann Rheum Dis 2007;66(6):790-797. doi:10.1136/ard.2006.065656
1. Silva-Barrios S, Charpentier T, Stäger S. The Deadly Dance of B Cells with Trypanosomatids. Trends Parasitol 2018;34(2):155-171. doi:10.1016/j.pt.2017.10.001
2. Gibson-Corley KN, Boggiatto PM, Bockenstedt MM, Petersen CA, Waldschmidt TJ, Jones

DE. Promotion of a Functional B Cell Germinal Center Response after Leishmania Species Co-Infection Is Associated with Lesion Resolution. AJPA 2012;180(5):2009-2017. doi:10.1016/j.ajpath.2012.01.012

3. Wanasen N, Xin L, Soong L. Pathogenic role of B cells and antibodies in murine Leishmania amazonensis infection. Int J Parasitol 2008;38:417-429. doi:10.1016/j.ijpara.2007.08.010
4. Shi Y, Agematsu K, Ochs HD, Sugane K. Functional analysis of human memory B-cell subpopulations: IgD+CD27+ B cells are crucial in secondary immune response by producing high affinity IgM. Clin Immunol 2003;108(2):128-37.
5. Kiyama K, Kawabata D, Hosono Y, et al. Serum BAFF and APRIL levels in patients with IgG4-related disease and their clinical significance. Arthritis Res Ther 2012;14(2):1-8. doi:10.1186/ar3810
6. Mackay F, Sierro F, Grey ST, Gordon TP. Cracking the BAFF code. Nat Rev Immunol 2009;9(7):491-502. doi:10.1038/nri2572.
7. Mackay F, Sierro F, Grey ST, Gordon TP. The BAFF/APRIL system: an important player in systemic rheumatic diseases. Curr Dir Autoimmun 2005;8:243–65.
8. Tangye SG, Bryant VL, Cuss AK, Good KL. BAFF, APRIL and human B cell disorders. Semin Immunol 2006;18(5):305-317.doi:10.1016/j.smim.2006.04.004
9. Fontaine J, Chagnon-Choquet J, Valcke HS, Poudrier J, Roger M. High expression levels of B lymphocyte stimulator (BLyS) by dendritic cells correlate with HIV-related B-cell disease progression in humans. Blood 2011;117(1):145-155. doi:10.1182/blood-2010-08-301887
10. Scholzen A, Teirlinck AC, Bijker EM, et al. BAFF and BAFF Receptor Levels Correlate with B Cell Subset Activation and Redistribution in Controlled Human Malaria Infection. J Immunol 2014;192(8):3719-3729. doi:10.4049/jimmunol.1302960
11. Portugal S, Tipton CM, Sohn H, et al. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. Elife 2015;1-21. doi:10.7554/eLife.07218
12. Borhis G, Trovato M, Chaoul N, Ibrahim HM, Richard Y. B-cell-activating factor and the B-cell compartment in HIV/SIV infection. Front Immunol 2017;8:1338. doi:10.3389/fimmu.2017.01338
13. Goto Y, Omachi S, Sanjoba C, Matsumoto Y. Short report: Elevation of serum B-cell

activating factor levels during visceral leishmaniasis. Am J Trop Med Hyg 2014;91(5):912-914. doi:10.4269/ajtmh.14-0260

14. David CV, Craft N. Cutaneous and mucocutaneous leishmaniasis. Dermatol Ther 2009;22(6):491-502. doi:10.1111/j.1529-8019.2009.01272.x
15. Goto H, Lindoso JA. Current diagnosis and treatment of cutaneous and mucocutaneous leishmaniasis. Expert Rev Anti Infect Ther 2010;8(4):419-433. doi:10.1586/eri.10.19
16. Barrio A, Mora MC, Ramos F, Moreno S, Samson R, Basombrío MA. Short report: Use of kDNA-based polymerase chain reaction as a sensitive and differentially diagnostic method of American Tegumentary Leishmaniasis in disease-endemic areas of northern Argentina. Am J Trop Med Hyg 2007;77(4):636-639. doi:10.1053/joca.2002.0851
17. Barrio A, García Bustos MF, Mora MC, Parodi C, Ramos F, Moreno S. Identificación por PS-PCR de especies de Leishmania y su correlación con características clínicas, epidemiológicas y terapéuticas en Salta, Argentina. Rev Argentina Salud Pública 2009;1(1):30-33.
18. Parodi C, García Bustos MF, Barrio A, et al. American tegumentary leishmaniasis: T-cell differentiation profile of cutaneous and mucosal forms—co-infection with Trypanosoma cruzi. Med Microbiol Immunol 2016;205(4):353-369. doi:10.1007/s00430-016-0455-0
19. Van der Poll T, Zijlstra EE, Mevissen M. Interleukin 6 during active visceral leishmaniasis and after treatment. Clin Immunol Immunopathol 1995;77(1):111-114.
20. Palazzi C, Buskila D, Angelo SD, Amico ED, Olivieri I. Autoimmunity Reviews Autoantibodies in patients with chronic hepatitis C virus infection : pitfalls for the diagnosis of rheumatic diseases. Autoimmun Rev 2012;11(9):659-663. doi:10.1016/j.autrev.2011.11.011
21. García Bustos MF, González Prieto G, Ramos F, et al. Clinical and epidemiological features of leishmaniasis in northwestern-Argentina through a retrospective analysis of recent cases. Acta Trop 2016;154:125-132. doi:10.1016/j.actatropica.2015.11.008
22. Asito AS, Piriou E, Jura WG, et al. Suppression of circulating IgD+CD27+ memory B cells in infants living in a malaria-endemic region of Kenya. Malar J 2011;10(1):362. doi:10.1186/1475-2875-10-362
23. Moura RA, Weinmann P, Pereira PA, et al. Alterations on peripheral blood B-cell subpopulations in very early arthritis patients. Rheumatology 2010;49(6):1082-1092. doi:10.1093/rheumatology/keq029

24. Jacobsen MC, Thiébaut R, Fisher C, et al. Pediatric Human Immunodeficiency Virus Infection and Circulating IgD $^{+}$ Memory B Cells. J Infect Dis 2008;198(4):481-485. doi:10.1086/590215
25. Mackay F, Schneider P, Rennert P, Browning J. BAFF and APRIL: A Tutorial on B Cell Survival. Annu Rev Immunol 2003;21(1):231-264. doi:10.1146/annurev.immunol.21.120601.141152
26. Scholz JL, Crowley JE, Tomayko MM, et al. BLyS inhibition eliminates primary B cells but leaves natural and acquired humoral immunity intact. Proc Natl Acad Sci 2008;105(40):15517-15522. doi:10.1073/pnas.0807841105
27. Salazar-Camarena DC, Ortiz-Lazareno PC, Cruz A, et al. Association of BAFF, APRIL serum levels, BAFF-R, TACI and BCMA expression on peripheral B-cell subsets with clinical manifestations in systemic lupus erythematosus. Lupus 2016;25(6):582-592. doi:10.1177/0961203315608254
28. Sellam J, Miceli-Richard C, Gottenberg JE, et al. Decreased B cell activating factor receptor expression on peripheral lymphocytes associated with increased disease activity in primary Sjögren's syndrome and systemic lupus erythematosus. Ann Rheum Dis 2007;66(6):790-797.
29. Nduati E, Gwela A, Karanja H, et al. The plasma concentration of the B cell activating factor is increased in children with acute malaria. J Infect Dis 2011;204(6):962-970. doi:10.1093/infdis/jir438
30. Montes CL, Acosta-Rodríguez EV, Mucci J, Zuniga EI, Campetella O, Gruppi A. A Trypanosoma cruzi antigen signals CD11b+ cells to secrete cytokines that promote polyclonal B cell proliferation and differentiation into antibody-secreting cells. Eur J Immunol 2006;36(6):1474-1485. doi:10.1002/eji.200535537
31. Oliveira WN, Ribeiro LE, Schrieffer A, Machado P, Carvalho EM, Bacellar O. The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of human tegumentary leishmaniasis. Cytokine 2014;66(2):127-132. doi:10.1016/j.cyto.2013.12.016.The
32. Bacellar O, Lessa H, Schriefer A, et al. Up-Regulation of Th1-Type Responses in Mucosal Leishmaniasis Patients. Infect Immun 2002;70(12):6734-6740. doi:10.1128/IAI.70.12.6734
33. Soni B, Saha B, Singh S. Cytokine Systems cues governing IL6 signaling in leishmaniasis. Cytokine 2018;106:169-175. doi:10.1016/j.cyto.2017.11.001
34. Pisetsky DS, Vrabie IA. Antibodies to DNA: infection or genetics? *Lupus* 2009;18:1176-

1180. doi:10.1177/0961203309106492

Figure legends

Fig. 1. Subtypes of peripheral memory B cells in cutaneous leishmaniasis (CL) and mucosal leishmaniasis (ML). The $CD19^+$ B cell population wasgated and analyzed for CD27 versus IgD in (A) naïve B cells, IgD^+CD27^-; (B) nonclass-switched memory B cells, IgD^+CD27^+; (C) class-switched memory B cells, IgD^-CD27^+; and (D) double-negativeB cells, IgD^-CD27^-. Four clinical groups were compared for each set of markers: healthy subjects, HS, white circles (n = 15); CL with single lesions, CL-S (n = 13) and CL with multiple lesions, CL-M (n = 6), light grey circles; ML, dark grey circles (n = 19). The CL and ML cases with detectable antibodies for *T. cruzi* are represented by half black circles. $*p < 0.05$, $**p < 0.01$, $****p < 0.0001$ using the Kruskal-Wallis test and Dunn`s post-test. Scatter dot plots, line and error bars show the median and the range.

Fig. 2. Gating strategy for the analysis of the $CD19^+$ B cell peripheral population. (A) Lymphocytes were gated by forward and side light scatter, and subsequently (B) the $CD19^+$ B cells were selected. (C) Representative dot plots from a healthy subject (HS), a cutaneous leishmaniasis (CL) patient and a mucosal leishmaniasis patient (ML) are shown. The graphs show IgD and CD27 staining of the gated $CD19^+$ B cells.

Fig. 3. Plasma concentrations of B-cell activating factor (BAFF) and total IgG in cutaneous (CL) and mucosal (ML) leishmaniasis. The amount of soluble factors was compared in six clinical groups: healthy subjects, HS, white circles (n = 13-24); CL with single lesions, CL-S (n = 19-22) and CL with multiple lesions, CL-M (n = 8-11), light grey circles; patients recovered from CL (R-CL, light grey squares, n = 7); ML, dark grey circles (n = 28-32) and patients recovered from ML (R-ML, dark grey squares, n = 7). CL and ML patients with detectable antibodies for *T. cruzi* are represented by half black circles or squares. (A) Plasma BAFF concentration; (B) total IgG concentration. Comparison among the HS, CL-S, CL-M and ML groups by Kruskal-Wallis test and Dunn`s post-test, $**p < 0.01$, $***p < 0.001$.Comparison of CL-M vs. R-CL and ML vs. R-ML by Mann Whitney U-test, $*p = 0.0022$ and $***p = 0.0003$, respectively. Scatter dot plots, line and error bars show the median and range.

Fig. 4. Plasma levels of B-cell activating factor (BAFF) and proinflammatory cytokines in mucosal leishmaniasis (ML). (A) BAFF levels in ML patients with detectable concentration of IFN-γ (ML IFNγ POS, n = 12) compared with ML patients with undetectable levels of IFN-γ (ML

IFNγ NEG, n = 8), Unpaired *t* test, *$p < 0.05$; (B) BAFF levels in ML patients with detectable concentration of IL-6 (ML IL6 POS, n = 11) compared with ML patients with undetectable levels of IL-6 (ML IL6 NEG, n = 5), Mann Whitney U-test, *$p < 0,05$. ML patients are represented by dark grey circles; ML patients with history of therapeutic failure are represented by light grey squares.

Fig. 5. Expression of the BAFF receptors on $CD27^{+}CD19^{+}$ B lymphocytes from patients with cutaneous (CL) and mucosal (ML) leishmaniasis. Four clinical groups were compared: healthy subjects, HS, white circles (n = 9-10); CL with single lesions, CL-S (n = 6-9) and CL with multiple lesions (n = 3-4) light grey circles; ML, dark grey circles (n = 8-12). CL and ML patients with detectable antibodies for *T. cruzi* are represented by half black circles. (A) Expression of BAFF-receptor (BAFF-R); (B) Expression of transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI). *$p < 0.05$; **$p < 0.01$ using Kruskal-Wallis test and Dunn`s post-test.Scatter dot plots, line and error bars show the median and range.

Table 1. Clinical features of patients with cutaneous and mucosal leishmaniasis

Clinical form	No. patients	Age (years)*	Male gender	*L. (V.) braziliensis*	*L. (L.) amazonensis*	Anti- *T. cruzi* antibodies	Lesion age*	History of therapeutic failure	Time of complete recovery*
Cutaneous leishmaniasis									
Single active lesion	25	30 (13-79)	14/25 (60 %)	13/15 (87 %)	2/15 (13 %)	5/25 (20 %)	3 (0.5–48) months	4/25 (16 %)	NA
Multiple active lesions	12	38 (19-80)	8/12 (67 %)	7/8 (87.5 %)	1/8 (12.5 %)	3/12 (25 %)	5 (2–36) months	2/12 (17 %)	NA
Clinical recovery	7	34 (15-40)	5/7 (70 %)	NA	NA	1/7 (14 %)	NA	NA	2 (2-36) months
Mucosal leishmaniasis									
Mucosal active lesions	40	48 (13-75)	32/40 (80 %)	9/11 (82 %)	2/11 (18 %)	17/40 (42.5 %)	3 (0.17-30) years	19/40 (48%)	NA
Clinical recovery	7	39 (13-75)	5/7 (71 %)	NA	NA	4/7 (57 %)	NA	NA	10 (10-22) months
Control group									
Healthy subjects	25	34 (22-68)	16/25 (64 %)	NA	NA	0/25 (0 %)	NA	NA	NA

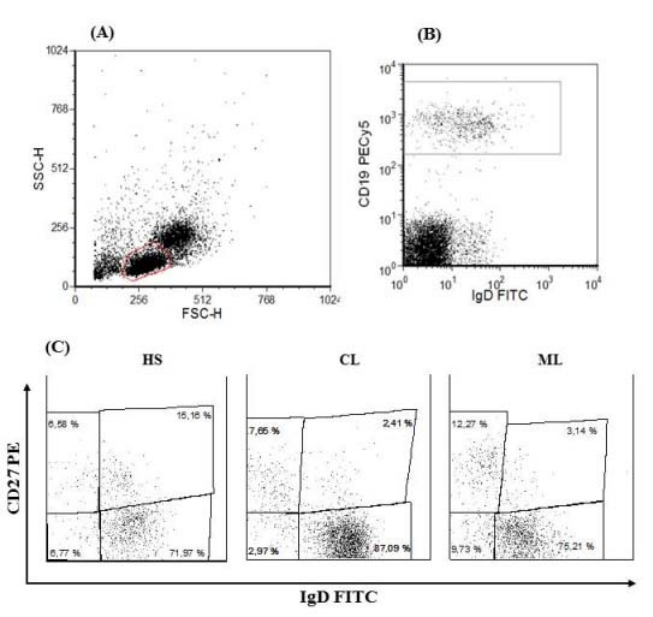
Note: NA, not applicable; *Median (Min-Max)

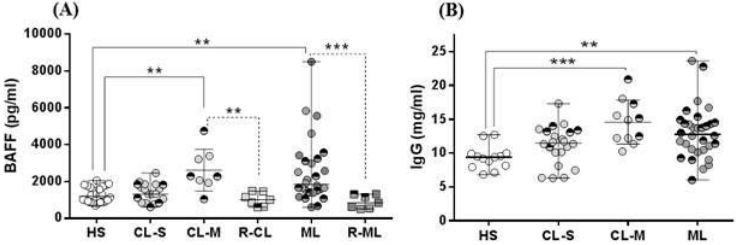
Table 2. Assessment of double-stranded DNA (dsDNA) antibodies in tegumentary leishmaniasis

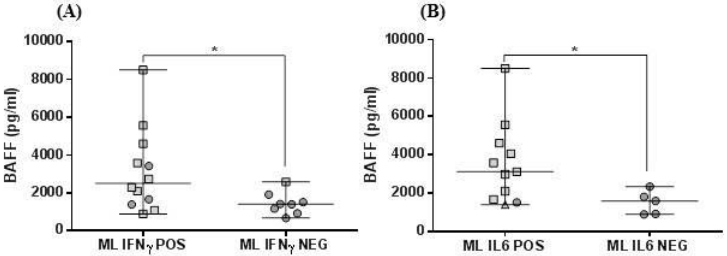
Patient groups	dsDNA antibodies	
	No. of subjects with negative findings (< 25 WHO-IU/ml)	**No. of subjects with positive findings (> 40 WHO-IU/ml)**
CL	17	0
CL with detectable antibodies for *T. cruzi*	5	0
ML	13	1
ML with detectable antibodies for *T. cruzi*	9	0
Total	**44**	**1**

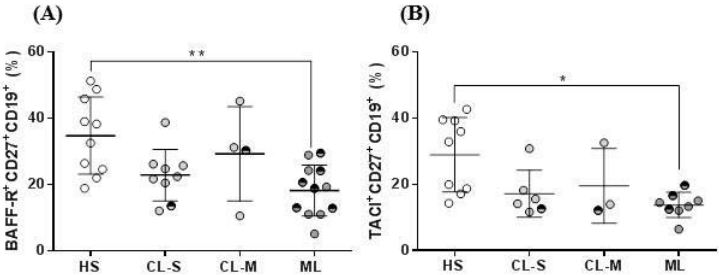
Note: CL, cutaneous leishmaniasis; ML, mucosal leishmaniasis

(A)
(B)
(C)
(D)
IgD+CD27+CD19+ (%)
HS
CL-S
CL-M
ML
**
*
****


(A)
1024
768
512
256
0
SSC-H
0
256
512
768
1024
FSC-H
(B)
10^4
10^3
10^2
10^1
10^0
CD19 PECy5
10^0
10^1
10^2
10^3
10^4
IgD FITC
(C)
HS
CL
ML
CD27 PE
6,58 %
15,16 %
6,77 %
71,97 %
7,65 %
2,41 %
2,97 %
87,09 %
12,27 %
3,14 %
9,73 %
75,21 %
IgD FITC


(A)
BAFF (pg/ml)
10000
8000
6000
4000
2000
0
**
**
**
***
HS
CL-S
CL-M
R-CL
ML
R-ML
(B)
IgG (mg/ml)
25
20
15
10
5
0
**
***
HS
CL-S
CL-M
ML


(A)
BAFF (pg/ml)
10000
8000
6000
4000
2000
0
*
ML IFNγ POS
ML IFNγ NEG
(B)
BAFF (pg/ml)
10000
8000
6000
4000
2000
0
*
ML IL6 POS
ML IL6 NEG


(A)
BAFF-R+ CD27+ CD19+ (%)
60
40
20
0
**
HS
CL-S
CL-M
ML
(B)
TACI+ CD27+ CD19+ (%)
60
40
20
0
*
HS
CL-S
CL-M
ML