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Original article

Characterization of the immune response to *Leishmania infantum* recombinant antigens

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Abstract

Leishmaniases have a high prevalence in tropical countries. In order to improve existing diagnostic systems based on total *Leishmania* proteins, and to identify antigen candidates for vaccine development, an intensive search for the identification of antigens was performed using molecular biology techniques. In this study, the immune response to three *L. infantum* recombinant antigens was evaluated. Upon stimulation with KMP11, mononuclear cells from leishmaniasis patients produced high levels of IL-10, while a predominant IFN-γ production could be observed in cultures stimulated with H2A and soluble *Leishmania* antigen. All the recombinant antigens induced very little IL-5. KMP11 decreased IFN-γ production by 48% in cultures of peripheral blood mononuclear cells from cutaneous leishmaniasis patients who had been stimulated with soluble *Leishmania* antigen. Furthermore, antibodies to KMP11 were detected in the sera from all patients with visceral leishmaniasis and in the majority of the sera from patients with cutaneous leishmaniasis or individuals with asymptomatic *L. chagasi* infection. Thus, KMP11 is recognized by cells and sera of patients with different clinical forms of leishmaniasis, and KMP11, through IL-10 production, proved to be a potent antigen in modulating type 1 immune response.

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Keywords: Leishmaniasis; Immuneregulation; IFN-γ; KMP11

1. Introduction

Leishmaniases are endemic in developing tropical countries, affecting ~600,000 people every year [1]. Due to the high cost of treatment, evidence of resistance and the toxicity generated by antimony therapy [2], the development of a vaccine must be considered an anticipated solution. Most studies aimed at identifying antigens from *Leishmania* sp. have searched for molecules with the ability to stimulate IL-2, IFN-γ and IL-12 [3–5], once the Th1 type immune response was known to be the major defense mechanism against *Leishmania* infection [6]. In fact, IFN-γ is the main cytokine implicated in the activation of macrophages to the killing of *Leishmania* [7]. The absence of a type 1 immune response to *Leishmania* antigen is documented in patients with visceral leishmaniasis and diffuse cutaneous leishmaniasis diseases characterized by parasite multiplication and dissemination [8,9]. However, in patients infected with *L. braziliensis*, the presence of strong IFN-γ and TNF-α production does not prevent development of the cutaneous or mucosal disease [10,11]. In fact, an exacerbated inflammatory response may be correlated with the tissue damage observed in cutaneous and mucosal leishmaniasis [11,12].

IL-10 is the main downregulatory cytokine of type 1 immune response inhibiting IL-12, IL-2, TNF-α and IFN-γ synthesis and decreasing macrophage activity [13–16]. Although high levels of IL-10 may be dangerous in patients with infections caused by intracellular pathogens, the modulation of an inflammatory response mediated by this cytokine

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is important for the regulation of a chronic inflammatory reaction [17,18]. In the present study, the immune response to three recombinant *L. infantum* antigens (H3, H2A and KMP11) was characterized in patients with cutaneous leishmaniasis and visceral leishmaniasis and in individuals with subclinical *L. chagasi* infection. The finding that one of these antigens (KMP11) induced high levels of IL-10 led us to determine the ability of KMP11 to modulate type 1 immune response observed in cutaneous leishmaniasis.

2. Material and methods

2.1. Patients

Cutaneous leishmaniasis patients (n = 13) were recruited from the health post of Corte de Pedra (southeast of Bahia state, Brazil), an area of *L. braziliensis* transmission, where clinical and immunological studies have been carried out for more than 20 years. All patients had active disease and were evaluated prior to therapy. Subclinical *L. chagasi*-infected individuals (n = 10) were recruited from the peri-metropolitan area of Natal, where a cohort study of *L. chagasi*-infected individuals has been followed for 5 years [19]. All persons with subclinical *L. chagasi* infection had antibodies to *Leishmania* antigen and had a positive delayed type hypersensitivity for more than 1 year without evidence of clinical symptoms related to kala-azar. Visceral leishmaniasis patients (n = 10) were admitted to the Hospital Universitário Prof. Edgard Santos, and diagnosis was confirmed by parasite isolation. Sera from visceral leishmaniasis patients were obtained prior to and after therapy. Healthy individuals (n = 20) were used as controls. The diagnosis of cutaneous leishmaniasis was performed by parasite isolation or by histopathological findings characteristic of cutaneous leishmaniasis and a positive skin test reaction with *Leishmania* antigen. Healthy employees (n = 20) living outside endemic areas of leishmania were used as controls. This study was approved by the ethical committee of the hospital, and informed consent was obtained from all patients.

2.2. Leishmania *antigens and antibody detection*

Two histones (H3 and H2A) and the 11-kDa surface protein (KMP11) were purified as previously described [20,21]. The leishmania species used in the present study were characterized by a World Health Organization center (MHOM). Soluble *Leishmania* antigen (SLA) was prepared from a strain of *L. amazonensis* (MHOM BR-86 BA-125) or from a strain of *L. chagasi* (MHOM BA-62) as previously described [22]. After a dose curve response had been performed, these recombinant antigens were used at a concentration of 10 µg/ml of culture.

An ELISA test was performed to detect antibodies against KMP11 using a 1:50 dilution of the sera as previously described for soluble *Leishmania* antigen [23]. The KMP11 antigen was used at a concentration of 100 ng/well. A serologic test for KMP11 antigen was considered positive when the OD was > 0.030, which represents the mean + three standard deviations of the OD observed when the sera of healthy subjects not exposed to *Leishmania* were used.

2.3. Cell culture and cytokine assay

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by Ficoll-Hypaque gradient centrifugation. After being washed three times in 0.9% NaCl, the PBMCs were resuspended in RPMI-1640 culture medium (GIBCO BRL, Grand Island, NY) supplemented with 10% human AB serum, 100 IU/ml of penicillin and 100 µg/ml of streptomycin. Cells were adjusted to 3×10^6 cells/ml, placed in 24-well plates and stimulated with SLA (1 µg/ml), H3 (10 µg/ml), H2A (10 µg/ml), KMP11 (10 µg/ml) or *L. chagasi* antigen (1 µg/ml). Some of the cells were stimulated with soluble *Leishmania* antigen plus KMP11. To evaluate the origin of IL-10, we determined the amount of this cytokine in cultures containing adherent and non-adherent cells and by determination of the ability of KMP11 to downregulate IFN-γ production in non-adherent cells. For these experiments, macrophages were depleted by adherence to a plastic surface as previously described [24]. Monoclonal antibody anti-IL-10 (200 µg/ml) (Coffman, DNAX Institute, Palo Alto, CA) was added to some SLA-stimulated cultures. Cultures of the whole population of cells, adherent and non-adherent, were incubated for 72 h at 37 °C and 5% CO_2. Supernatants were collected and stored at –70 °C. The levels of IL-5, IL-10 and IFN-γ were measured by the ELISA sandwich method using reagents (R&D Systems, Minneapolis, MN). The results are expressed in pg/ml.

2.4. Statistical analysis

The statistical analysis was performed using the Mann Whitney test.

3. Results

The levels of IFN-γ, IL-5 and IL-10 in supernatants of PBMC cultures from cutaneous leishmaniasis patients in response to SLA and the recombinant antigens H3, H2A and KMP11 are shown in Fig. 1 . IFN-γ levels in cultures stimulated with H3 and H2A antigen were 234 ± 576 and 252 ± 429 pg/ml, respectively. These levels were lower than those observed in cultures stimulated with SLA (1003 ± 564 pg/ml). Low levels of IFN-γ (104 ± 120 pg/ml) were detected in 11 of the 13 (84%) supernatants of PBMC cultures stimulated with KMP11. IL-5 levels were significantly higher in supernatants of SLA-stimulated cultures than in those stimulated with recombinant antigens. In contrast with what was observed in the supernatants of PBMC of H2A- or SLA-stimulated cultures from cutaneous leishmaniasis pa-

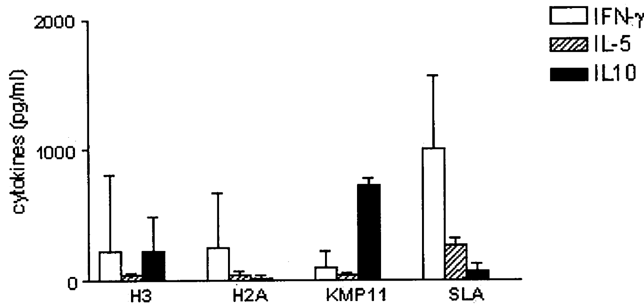

Fig. 1. IFN-γ, IL-5 and IL-10 levels in PBMCs from cutaneous leishmaniasis patients ($n = 13$) in response to H3 (10 µg/ml), H2A (10 µg/ml), KMP11 (10 µg/ml) and *L. amazonensis* (SLA) (1 µg/ml).

tients, in which production of IL-10 was absent or low, high levels of IL-10 were observed in KMP1-stimulated cultures (725 ± 54 pg/ml) and in the majority of the supernatants of H3-stimulated cultures (231 ± 157 pg/ml).

IFN-γ, IL-5 and IL-10 levels in the supernatants of PBMC cultures from six individuals with subclinical *L. chagasi* infection are shown in Fig. 2 . IFN-γ production predominated over that of IL-5 and IL-10 in these supernatants after stimulation of cultures with H3 or H2A. Although KMP11 induced higher ($P < 0.05$) levels of IL-10 (139 ± 121 pg/ml) than those observed after stimulation with SLA (24 ± 51 pg/ml), the amount of IL-10 was much lower ($P < 0.01$) than that observed in the PBMC cultures from cutaneous leishmaniasis patients. Only KMP11 was able to induce high levels of IL-10 on PBMC cultures of healthy subjects (669 ± 195 pg/ml).

Since a high production of IL-10 was observed after KMP11 stimulation, the ability of this antigen to modulate the Th1 type immune response in cutaneous leishmaniasis patients was tested in cell cultures after SLA stimulation (Fig. 3). The addition of KMP11 to SLA-stimulated cultures decreased the IFN-γ production from 1140 ± 761 to 441 ± 496 pg/ml ($P < 0.01$).

To confirm that the downregulation of IFN-γ was dependent on the high levels of IL-10 induced by KMP11, the neutralization of this cytokine was performed in cultures stimulated with KMP11 plus SLA; in four cutaneous leishmaniasis patients, addition of anti-IL-10 enhanced the IFN-γ production from 207 ± 212 to 728 ± 518 pg/ml (Fig. 4).

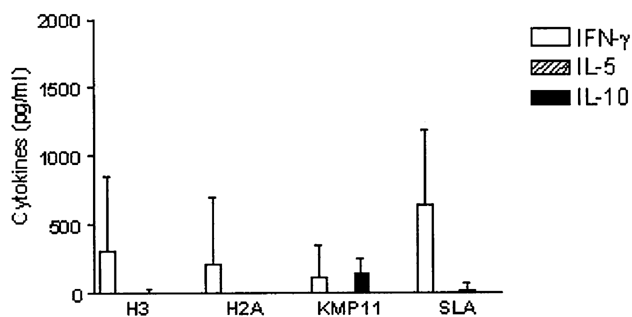

Fig. 2. IFN-γ, IL-5 and IL-10 levels in PBMCs from asymptomatic *L. chagasi*-infected patients ($n = 6$) in response to H3, H2A, KMP11 and *L. chagasi* (SLA) antigen.

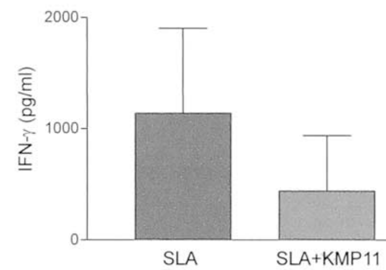

Fig. 3. Ability of KMP11 to suppress IFN-γ production in SLA-stimulated lymphocyte cultures from cutaneous leishmaniasis patients.

Two analyses were performed to determine the source of IL-10. First, we evaluated whether the suppression of IFN-γ production mediated by KMP11 in cultures stimulated with *Leishmania* antigen could be observed in the population of non-adherent cells. We also determined the production of IL-10 in cultures of adherent as well as non-adherent cells and in cultures of adherent cells stimulated with KMP11. Depletion of adherent cells abrogates the ability of KMP11 to suppress IFN-γ production (Fig. 5). While addition of KMP11 to PBMC cultures stimulated with *Leishmania* antigen suppressed IFN-γ production by 74%, no suppression of

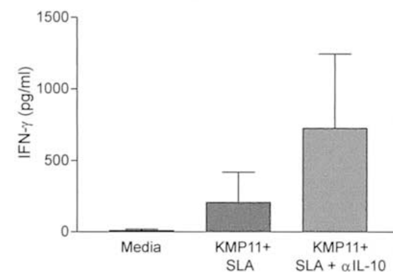

Fig. 4. Anti-IL-10 monoclonal antibody (50 µg/ml) enhanced IFN-γ production in SLA (1 µg/ml) plus KMP11 (10 µg/ml)-stimulated cultures from four cutaneous leishmaniasis patients.

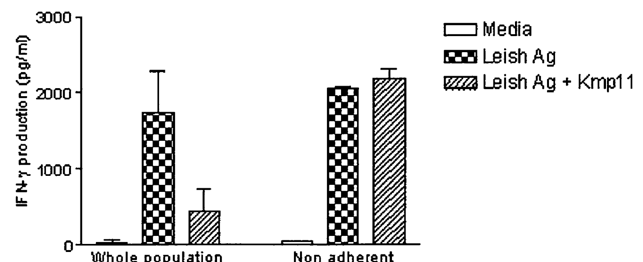

Fig. 5. Evaluation of the ability of KMP11 (10 µg/ml) to downregulate IFN-γ production in SLA-stimulated PBMCs (whole population) and in cells depleted of macrophages (non-adherent cells) of cutaneous leishmaniasis patients.

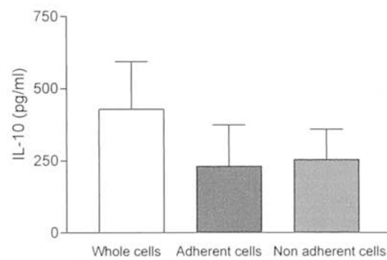

Fig. 6. IL-10 levels in KMP11-stimulated cultures of different cell populations from cutaneous leishmaniasis patients ($n = 4$).

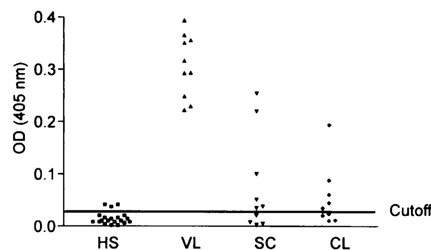

Fig. 7. Anti-KMP11 IgG titers of healthy subjects (HS), visceral leishmaniasis patients (VL), subclinical *L. chagasi*-infected individuals (SC) and cutaneous leishmaniasis patients (CL).

IFN-γ production was observed when KMP11 was added to cultures of non-adherent cells stimulated with *Leishmania*antigen (Fig. 5). It should be noted that while the percentage of macrophages in PBMC cultures as measured by esterase staining was 24 ± 5% in the mononuclear cells, it fell to 2 ± 1.8% in the non-adherent cell population. When IL-10 was measured in supernatants of adherent and non-adherent KMP11-stimulated cells, no significant difference was detected (Fig. 6).

Antibody levels to KMP11 were also determined in the serum samples from visceral leishmaniasis, subclinical *L. chagasi* infection, cutaneous leishmaniasis and healthy subjects (Fig. 7). Anti-KMP11 IgG antibodies were observed in serum samples of all visceral leishmaniasis patients and in sera of some individuals with subclinical *L. chagasi* infection and cutaneous leishmaniasis patients. Antibodies to KMP11 were not detected in the majority of sera from healthy subjects not exposed to leishmania infection.

4. Discussion

The search for parasite antigens able to induce an immune response has been predominantly associated with the identification of proteins that may be used for serodiagnosis or vaccine development [3–5]. In this study, we characterized the immune response to three *L. infantum* recombinant antigens that were recognized by sera of visceral leishmaniasis and cutaneous leishmaniasis patients. We found that the KMP 11-kDa antigen induced high levels of IL-10 in PBMC cultures of patients with cutaneous leishmaniasis, in individuals with asymptomatic *L. chagasi* infection and in healthy subjects. Characterization of the cellular immune response was performed in patients with cutaneous leishmaniasis and in individuals with subclinical *L. chagasi* infection, since it is known that a T-cell response develops when cells from these individuals are stimulated with SLA [25,26]. Although the three antigens were to some extent able to stimulate PBMCs from these individuals, the response was quite variable. The three antigens induced lower amounts of IFN-γ and IL-5 than did SLA. Additionally, the number of individuals who produced these cytokines upon stimulation with the recombinant antigens was quite variable. In contrast, there was a high production of IL-10 in KMP11-stimulated cultures in 18 of 19 individuals with leishmaniasis. This antigen induced a higher level of IL-10 in PBMCs from patients with cutaneous leishmaniasis than in PBMCs from individuals with an *L. chagasi* infection. The documentation that KMP11 induces IL-10 in PBMCs from healthy subjects suggests that this cytokine is produced by macrophages or that KMP11-like epitopes may be found in other antigens, thus making the majority of the subjects respond to it. Our data suggest that the source of IL-10 can be both macrophages and lymphocytes, since macrophage depletion abrogated the ability of KMP11 to suppress IFN-γ production and because of the finding that IL-10 was produced by both the adherent and non-adherent cells. Additionally, the explanation for finding different levels of IL-10 in patients with cutaneous leishmaniasis and those who had subclinical *L. chagasi* infection would be the participation of lymphocytes in IL-10 secretion. It cannot be ruled out that macrophages from different individuals produce different amounts of IL-10 after stimulation with KMP11. The observation of antibodies against KMP11 only in patients infected with *Leishmania* is evidence that this antigen induces an acquired immune response.

By modulating macrophages and T-cell functions, IL-10 is the major regulatory cytokine of the inflammatory and type 1 immune response [14]. In diseases caused by intracellular parasites, high production of IL-10 may be dangerous to the host due to its ability to decrease type 1 immune response and macrophage activation. This would allow multiplication of the parasites, as we observed in patients with visceral leishmaniasis, and diffusion of cutaneous leishmaniasis [27,28]. However, IL-10 may be crucial in modulating the inflammatory response associated with tissue damage. In fact, in patients with cutaneous and mucosal leishmaniasis due to *L. braziliensis*, the high type 1 immune response, when not appropriately modulated, may cause tissue damage [11,12]. Modulation of the immune response by IL-10 has also been suggested to protect animals from tissue destruction in autoimmune diseases such as diabetes and experimental acute immune encephalitis [29,30]. We showed that KMP11 was able to downregulate IFN-γ production in patients with cuta-

neous leishmaniasis and that this effect is dependent on the modulation of IL-10, since neutralization of IL-10 by monoclonal antibody abrogates the regulatory effect of this antigen. Since KMP11 is predominantly expressed in promastigotes and only expressed at very low levels in amastigotes [31], it is unlikely that this antigen is detrimental to the host by significantly decreasing type 1 immune response needed for parasite control. The observation that exogenous KMP11 downregulates IFN-γ production indicates that this antigen may play a role in modulating the exaggerated type 1 immune response observed in chronic inflammatory diseases.

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References

[1] P. Desjeux, Leishmaniasis. Public health aspects and control, Clin. Dermatol. 14 (1996) 417–423.

[2] P.D. Marsden, R.N. Sampaio, E.M. Carvalho, J.P. Veiga, J.L. Costa, E.A. Llanos-Cuentas, High continuous antimony therapy in two patients with unresponsive mucosal leishmaniasis, Am. J. Trop. Med. Hyg. 34 (1985) 710–713.

[3] J.M. Burns Jr, J.M. Scott, E.M. Carvalho, D.M. Russo, C.J. March, K.P. Van Ness, S.G. Reed, Characterization of a membrane antigen of *Leishmania amazonensis* that stimulates human immune responses, J. Immunol. 146 (1991) 742–748.

[4] Y.A. Skeiky, M. Kennedy, D. Kaufman, M.M. Borges, J.A. Guderian, J.K. Scholler, P.J. Ovendale, K.S. Picha, P.J. Morrissey, K.H. Grabstein, A. Campos-Neto, S.G. Reed, LeIF: a recombinant Leishmania protein that induces an IL-12-mediated Th1 cytokine profile, J. Immunol. 161 (1998) 6171–6179.

[5] D.M. Russo, J.M. Burns Jr, E.M. Carvalho, R.J. Armitage, K.H. Grabstein, L.L. Button, W.R. McMaster, S.G. Reed, Human T cell responses to gp63, a surface antigen of Leishmania, J. Immunol. 147 (1991) 3575–3580.

[6] P. Scott, P. Natovitz, R.L. Coffman, E. Pearce, A. Sher, Immunoregulation of cutaneous leishmaniasis. T cell lines that transfer protective immunity or exacerbation belong to different T helper subsets and respond to distinct parasite antigens, J. Exp. Med. 168 (1988) 1675–1684.

[7] H.W. Murray, B.Y. Rubin, C.D. Rothermel, Killing of intracellular *Leishmania donovani* by lymphokine-stimulated human mononuclear phagocytes. Evidence that interferon-gamma is the activating lymphokine, J. Clin. Invest. 72 (1983) 1506–1510.

[8] E.M. Carvalho, W.D. Johnson, E. Barreto, P.D. Marsden, J.L. Costa, S. Reed, H. Rocha, Cell mediated immunity in American cutaneous and mucosal leishmaniasis, J. Immunol. 135 (1985) 4144–4148.

[9] C. Pirmez, M. Yamamura, K. Uyemura, M. Paes-Oliveira, F. Conceicao-Silva, R.L. Modlin, Cytokine patterns in the pathogenesis of human leishmaniasis, J. Clin. Invest. 91 (1993) 1390–1395.

[10] M. Castes, D. Trujillo, M.E. Rojas, C.T. Fernandez, L. Araya, M. Cabrera, J. Blackwell, J. Convit, Serum levels of tumor necrosis factor in patients with American cutaneous leishmaniasis, Biol. Res. 26 (1993) 233–238.

[11] A. Ribeiro-de-Jesus, R.P. Almeida, H. Lessa, O. Bacellar, E.M. Carvalho, Cytokine profile and pathology in human leishmaniasis, Braz. J. Med. Biol. Res. 31 (1998) 143–148.

[12] H.A. Lessa, P. Machado, F. Lima, A.A. Cruz, O. Bacellar, J. Guerreiro, E.M. Carvalho, Successful treatment of refractory mucosal leishmaniasis with pentoxifylline plus antimony, Am. J. Trop. Med. Hyg. 65 (2001) 87–89.

[13] D.F. Fiorentino, M.W. Bond, T.R. Mosmann, Two types of mouse T helper cell. IV. Th2 clones secrete a factor that inhibits cytokine production by Th1 clones, J. Exp. Med. 170 (1989) 2081–2095.

[14] D.F. Fiorentino, A. Zlotnik, T.R. Mosmann, M. Howard, A. O'Garra, IL-10 inhibits cytokine production by activated macrophages, J. Immunol. 147 (1991) 3815–3822.

[15] R. de Waal Malefyt, J. Abrams, B. Bennett, C.G. Figdor, J.E. de Vries, Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes, J. Exp. Med. 174 (1991) 1209–1220.

[16] O. Bacellar, A. D'Oliveira Jr, S. Jeronimo, E.M. Carvalho, IL-10 and IL-12 are the main regulatory cytokines in visceral leishmaniasis, Cytokine 12 (2000) 1228–1231.

[17] J.W. Cook, S. Karakozis, D. Kim, H. Provido, E. Gongora, J.R. Kirkpatrick, Interleukin-10 attenuates proinflammatory cytokine production and improves survival in lethal pancreatitis, Am. Surg. 67 (2001) 237–241 discussion 241–232.

[18] S.A. Rezende, D.N. Silva-Teixeira, S.C. Drummond, A.M. Goes, IL-10 plays a role in the modulation of human granulomatous hypersensitivity against Schistosoma mansoni eggs induced by immune complexes, Scand. J. Immunol. 46 (1997) 96–102.

[19] S.M. Jeronimo, M.J. Teixeira, A. Sousa, P. Thielking, R.D. Pearson, T.G. Evans, Natural history of Leishmania (Leishmania) chagasi infection in Northeastern Brazil: long-term follow-up, Clin. Infect. Dis. 30 (2000) 608–609.

[20] M. Soto, J.M. Requena, L.C. Gomez, I. Navarrete, C. Alonso, Molecular characterization of a Leishmania donovani infantum antigen identified as histone H2A, Eur. J. Biochem. 205 (1992) 211–216.

[21] M. Soto, J.M. Requena, G. Morales, C. Alonso, The *Leishmania infantum* histone H3 possesses an extremely divergent N-terminal domain, Biochim. Biophys. Acta 1219 (1994) 533–535.

[22] S.G. Reed, R. Badaro, H. Masur, E.M. Carvalho, R. Lorenco, A. Lisboa, R. Teixeira, W.D. Johnson Jr, T.C. Jones, Selection of a skin test antigen for American visceral leishmaniasis, Am. J. Trop. Med. Hyg. 35 (1986) 79–85.

[23] R. Badaro, S.G. Reed, A. Barral, G. Orge, T.C. Jones, Evaluation of the micro enzyme-linked immunosorbent assay (ELISA) for antibodies in American visceral leishmaniasis: antigen selection for detection of infection-specific responses, Am. J. Trop. Med. Hyg. 35 (1986) 72–78.

[24] E.M. Carvalho, O. Bacellar, A. Barral, R. Badaro, W.D. Johnson Jr, Antigen-specific immunosuppression in visceral leishmaniasis is cell mediated, J. Clin. Invest. 83 (1989) 860–864.

[25] E.M. Carvalho, R. Badaro, S.G. Reed, T.C. Jones, W.D. Johnson Jr, Absence of gamma interferon and interleukin 2 production during active visceral leishmaniasis, J. Clin. Invest. 76 (1985) 2066–2069.

[26] E.M. Carvalho, A. Barral, D. Pedral-Sampaio, M. Barral-Netto, R. Badaro, H. Rocha, W.D. Johnson Jr, Immunologic markers of clinical evolution in children recently infected with Leishmania donovani chagasi, J. Infect. Dis. 165 (1992) 535–540.

[27] D.L. Sacks, S.L. Lal, S.N. Shrivastava, J. Blackwell, F.A. Neva, An analysis of T cell responsiveness in Indian kala-azar, J. Immunol. 138 (1987) 908–913.

[28] G. Bomfim, C. Nascimento, J. Costa, E.M. Carvalho, M. Barral-Netto, A. Barral, Variation of cytokine patterns related to therapeutic response in diffuse cutaneous leishmaniasis, Exp. Parasitol. 84 (1996) 188–194.

[29] M.E. Pauza, H. Neal, A. Hagenbaugh, H. Cheroutre, D. Lo, T-cell production of an inducible interleukin-10 transgene provides limited protection from autoimmune diabetes, Diabetes 48 (1999) 1948–1953.

[30] J.S. Yang, L.Y. Xu, Y.M. Huang, P.H. Van Der Meide, H. Link, B.G. Xiao, Adherent dendritic cells expressing high levels of interleukin-10 and low levels of interleukin-12 induce antigen-specific tolerance to experimental autoimmune encephalomyelitis, Immunology 101 (2000) 397–403.

[31] M.C. Thomas, M.V. Longobardo, E. Carmelo, C. Maranon, L. Planelles, M.E. Patarroyo, C. Alonso, M.C. Lopez, Mapping of the antigenic determinants of the T. cruzi kinetoplastid membrane protein-11. Identification of a linear epitope specifically recognized by human Chagasic sera, Clin. Exp. Immunol. 123 (2001) 465–471.