Evaluation of the expression of interleukin 1 beta (IL-1β) and interleukin 1 receptor antagonist (IL-1Ra) in leprosy patients

Avaliação da expressão de interleucina 1 beta (IL-1β) e antagonista do receptor de interleucina 1 (IL-1Ra) em pacientes com hanseníase

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ABSTRACT

Leprosy is an infectious and contagious spectral disease accompanied by a series of immunological events triggered by the host's response to the etiologic agent, *Mycobacterium leprae*. Evidence suggests that the induction and maintenance of the immune/inflammatory response in leprosy are linked to multiple cell interactions and soluble factors, mainly through the action of cytokines. The ELISA test was used to measure the levels of IL-1β and IL-1Ra in 37 new leprosy patients followed-up during treatment and 30 healthy controls. Peripheral blood was collected four times during the treatment of leprosy patients (MDT pretreatment, 2nd dose, 6th dose and post-MDT), and only once from the controls. The comparison of molecular levels in pre-MDT patients and controls showed a statistically significant difference for IL-1β. The results suggest the participation of this cytokine in the genesis of the immune/inflammatory process.

Key-words: Leprosy. Interleukins. IL-1β and IL-1Ra.

RESUMO

A hanseníase é uma doença infectocontagiosa espectral que acompanha-se por uma série de eventos imunológicos desencadeados pela resposta do hospedeiro frente ao agente etiológico, o *Mycobacterium leprae*. Evidências sugerem que a indução e manutenção da resposta imune/inflamatória na hanseníase estão vinculadas a interações de múltiplas células e fatores solúveis, particularmente através da ação de citocinas. Nesse estudo, foram mensurados níveis de IL-1β e IL-1Ra de 37 casos novos de hanseníase acompanhados ao longo do tratamento e 30 controles sadios pelo teste ELISA. A coleta de sangue periférico foi realizada em quatro tempos para os casos de hanseníase (pré-tratamento com PQT, 2ª dose, 6ª dose e pós-PQT) e em único momento para os controles. Na comparação dos níveis das moléculas de casos no pré-PQT e controles, houve diferença estatisticamente significativa somente para IL-1β. Nossos resultados sugerem a participação dessa citocina no processo imune/inflamatório.

Palavras-chaves: Hanseníase. Interleucinas. IL-1β e IL-1Ra.

Leprosy is a chronic infectious and parasitic disease caused by *Mycobacterium leprae*, an atoxic, resistant, acid-alcohol-fast, obligate intracellular bacillus, which induces an extraordinary cell-mediated immune response in diseased individuals and affects mainly the skin and branches of peripheral nerves[4 17 19 20]. Neural injury, recognized by many authors as the most serious complication of leprosy, is triggered by infection and accompanied by a series of immunological events, whose evolution sequelae often last many years after infection has ceased[22 28]. It presents an important peculiarity to clinicians and immunologists: the host's diversity in responding to its etiologic agent imposes a diagnostic challenge and an excellent model for understanding cellular immunity in humans[23].

Leprosy is characterized by its high infectivity and low pathogenicity; moreover, more than 95% of the population are naturally immune to such an infection[18 31 32 33]. This condition can be changed in function of the relationship between the agent, the environment and the host[10]. It is a spectrum disease characterized by contrasting clinical forms and the outcome of infection seems to depend on the predominant underpopulation of T lymphocytes, on

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macrophage activation and on when and how a certain cytokine is available at the site of the parasite[9 21 24 26].

In tuberculoid (TT) lesions, predominance of Th1 response and type 1 cytokines occurs, including interleukin (IL) 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α)[13 29]. These molecules are among the most studied cytokines and evidence indicates that both can act synergically to prevent bacillary proliferation, but they can also become pathogens, causing skin and neural lesions in the absence of regulating factors[2 5 7 8 25]. Thus, the decrease in the synthesis or blockage of the effects of a superproduction of such cytokines can particularly provide an option for the control of certain inflammatory disorders[11]. IL-1β, for example, is one of the molecular forms of IL-1 produced by practically all nucleated cell types (mainly monocytes, macrophages and dendritic cells) and it is among the most important cytokine-induced markers of inflammatory response[6]. The mechanism of natural inhibition of this cytokine involves the blockage of receptor binding by cytokine receptor antagonists, such as IL-1 Ra, an IL-1 receptor antagonist. IL-1 Ra is a protein of the interleukin family, originally described as a molecule secreted by monocytes and macrophages, which modulates a variety of IL-1-related immune and inflammatory responses[3].

In lepromatous (LL) lesions, on the other hand, predominance of Th2 response and type 2 cytokines occurs, including IL-4, IL-5 and IL-10. The *borderline* forms, in turn, represent a clinical and immunological pattern of intermediate response and the cytokines expressed both *in vitro* and *in vivo* cannot be related to any pattern already described[2 5 7 8 25].

Despite scientific advances in leprosy, many issues have yet to be investigated regarding immunology[14] and it is important to highlight that the induction and maintenance of the immune/inflammatory response are linked to multiple cell interactions and soluble factors, particularly by cytokine action. However, T lymphocyte activation and cytokine production cannot be evaluated in isolation during infection[23]. Therefore, the study of immunological processes is essential for understanding the mechanisms of the development and continuance of leprosy[9].

The purpose of this paper was to study the expression of IL-1β inflammatory cytokine and the IL-1Ra anti-inflammatory molecule in leprosy patients assisted by the Reference Center of Sanitary Dermatology in the city of Belo Horizonte, State of Minas Gerais, Brazil.

MATERIAL AND METHODS

This is an exploratory, descriptive, longitudinal and analytical study involving 37 new leprosy cases followed-up during multidrug therapy (MDT) and 30 noninfected subjects from an endemic area, here considered as healthy controls, conducted at the Dermatology Ambulatory of the Eduardo de Menezes Hospital Sanitary Dermatology Reference Center of the Minas Gerais State Foundation (FHEMIG), in Belo Horizonte, Brazil, from May 2006 to December 2007.

Data collection was initiated after the project was approved by the Ethics in Research Committee of the Eduardo de Menezes Hospital and of the Santa Casa de Misericordia de Belo Horizonte. All the participants agreed to sign a term of free informed consent.

Patients' medical records were used to assess the following variables: sex, age, number of skin and nerve lesions, bacilloscopy, ML-Flow serological test, Madrid protocol and operational classifications. The subjects' peripheral blood (more specifically, their plasma) and the Sandwich ELISA technique were used to measure the levels of IL-1β and IL-1Ra. In cases of leprosy, blood collection occurred four times during treatment (pretreatment with MDT, 2nd dose, 6th dose and post-MDT); in healthy controls it occurred once. However, the number of patients submitted to peripheral blood collection on the 2nd and 6th dose was lower than patients in the pre and posttreatment, 15 and 19 patients, respectively. The samples were then sent to the Federal University of Minas Gerais, Institute of Biological Sciences, Immunopharmacology Laboratory, where the experiments were conducted according to a standard protocol. The levels of the molecules studied were expressed in pg/ml.

For data analyses, the following methods were used: measures of central tendency and variability; the Mann-Whitney test for comparing molecular levels of IL-1β and IL-1Ra of the pretreatment stage and healthy controls; the Wilcoxon[1] test for comparing the molecular levels studied in the leprosy cases between one another (pre-MDT and the other treatment stages) and the Generalized Linear Model for repeated measures with four factors (F test)[15] for the longitudinal comparison of the IL-1β and IL-1Ra levels at the different treatment stages or phases. One limitation of the last analysis was the sample size, since the molecules were assessed at different stages (pre-MDT, 2nd dose, 6th dose and post-MDT) in only seven patients. In addition, for molecules that were significant in the comparison of leprosy cases in the pretreatment stage and healthy controls, a ROC (Receiver Operating Characteristic)[34] curve was used to characterize the best cutoff point of the measurements analyzed for the prediction of a positive leprosy case. The significance level adopted was 5%.

RESULTS

The results showed that most patients were male, with a mean age of approximately 50 years-old, with more than five skin lesions and more than one nerve affected, classified as *borderline* by the Madrid classification and as multibacillary (MB) by the operational classification; 37.8% of the cases were bacilloscopy positive and 64.9% were positive for the ML-Flow serological test.

Comparison of the molecular levels in leprosy cases in the pretreatment stage and in a healthy controls showed statistically significant difference ($p<0.05$) for IL-1β only (**Figure 1**), while comparison of molecular levels in leprosy cases at the pretreatment stage and the other stages (2nd dose, 6th dose and post-MDT) showed no statistically significant difference for any molecule. The F test also revealed no statistically significant difference and the graphic record showed a tendency for increase of the marginal mean of IL-1β levels starting from the 6th dose (referring to the 3rd phase, as observed in **Figure 2**). In addition, according to the ROC curve, patients with IL-1β levels greater than the established cutoff point (0.015) would be classified as probable leprosy cases with 75.7% sensitivity and 70% specificity (**Figure 3**).

The levels of IL-1Ra were undetectable in cases of leprosy and controls.

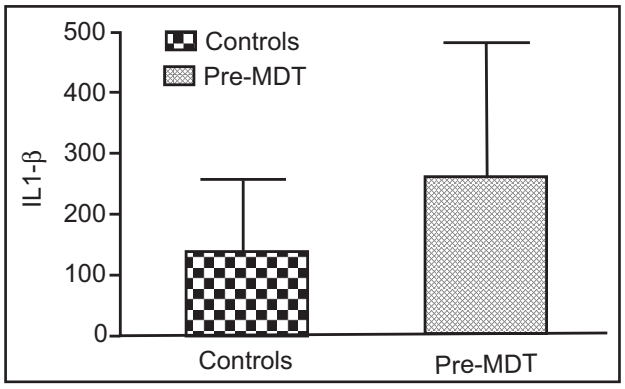

FIGURE 1

Comparison of the levels of IL-1β in cases of leprosy in the pretreatment stage and healthy controls, Belo Horizonte, May of 2006 to December of 2007.

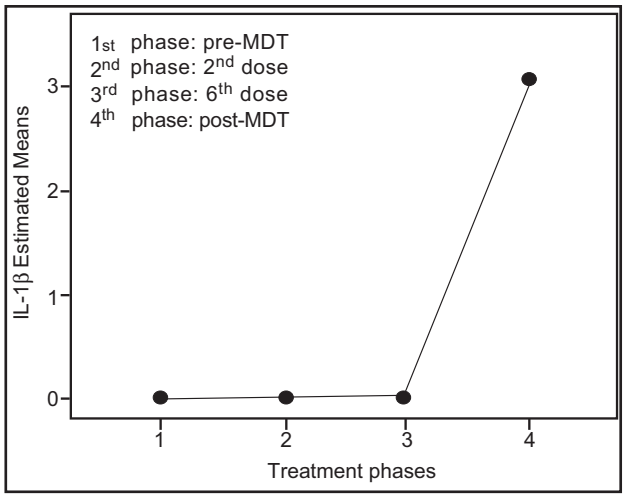

FIGURE 2

Graphic representation of the averages of IL-1β in the four phases of the study of the seven cases of leprosy assisted in the Service of Reference in Sanitary Dermatology of the Hospital Eduardo of Menezes - FHEMIG, Belo Horizonte, May of 2006 to December of 2007.

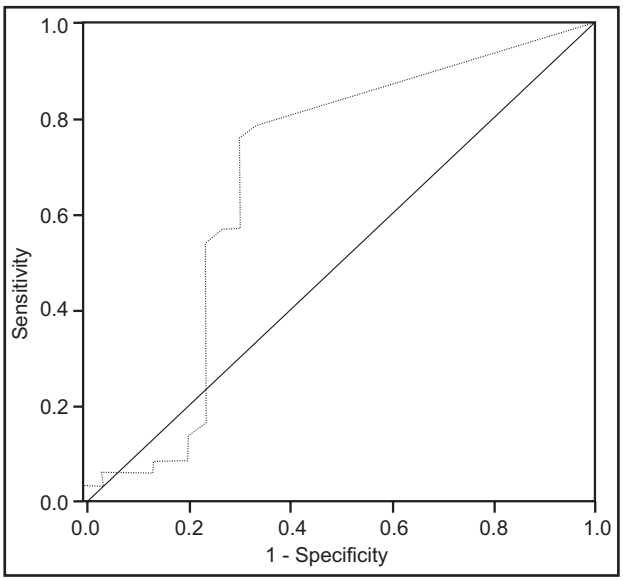

FIGURE 3

ROC Curve for the measures of IL-1β in the pre treatment as prediction of cases of leprosy *versus* healthy controls, Belo Horizonte, May of 2006 to December of 2007.

DISCUSSION

In this paper, the IL-1β levels revealed a statistically significant difference when the pretreatment leprosy cases and control group were compared. This finding agrees with that observed by Moubasher *et al*[16], in which nontreated leprosy patients exhibited significantly higher IL-1β serum levels and other molecules (IFN -γ, IL-2R, IL-10 and TNF-α) detected by the ELISA test compared to healthy controls. Therefore, we may deduce that IL-1β release in leprosy cases is an indication of the activation of a cell-mediated immune response to the bacillus, according to the observation of Watson *et al*[35], who quantified the production of IL-1β and IL-2 by mononuclear adhering cells from the peripheral blood of patients with different forms of leprosy.

The present study showed no statistically significant difference in IL-1β levels when comparing leprosy cases in pretreatment and other stages (2nd dose, 6th dose and post-MDT). These findings show that the respective cytokine levels were already altered before the onset of treatment in function of the bacillary load and no significant serum alterations were identified during multidrug therapy. The massive destruction and reduction of viable bacilli probably initiates the release of antigenic fractions, which would cause a persistent stimulus with consequent immune and inflammatory hyperreactivity[5 12 29 30]. Sarno *et al*[27] identified significantly higher IL-1β serum levels in all patients with *borderline tuberculoid* forms who showed no reaction, independent of the chemotherapy duration. They also detected high levels of the cytokine in the serum of leprosy patients with erythema nodosum leprosum (ENL).

In relation to the longitudinal comparison of IL-1β levels, no statistically significant difference was observed at the different stages of the study (pre-MDT, 2nd dose, 6th dose and post-MDT), and the graphic representation showed a tendency for an increase in the marginal mean of IL-1β levels starting from the 6th dose. This finding disagrees with that of Moubascher *et al*[16], who demonstrated a significant reduction in IL-1β serum levels and other molecules (IL-2R and IL-10) after one year of treatment in 36 leprosy cases. However, for this analysis, the present sample consisted of only seven patients, being considered too small for the researchers to make inferences. Nevertheless, the present results suggest that new investigations are still required to determine the effect of prolonged antimicrobial therapy on IL-1β production capability, an issue previously discussed[35].

Regarding IL-1β levels, the ROC curve showed a moderate prediction power to distinguish a positive leprosy case, with 75.7% sensitivity and 70% specificity.

The dosage of IL-1Ra, an anti-inflammatory molecule that selectively inhibits the effects of IL-1 by competing with the cell surface IL-1 receptor, the negative results obtained for both the controls and leprosy cases at the various stages of treatment could be justified by the fact that the levels were lower than the concentrations detected by the ELISA test.

Concerning the general limitations of this study, it is worth highlighting the loss of patients during follow-up, which prevented the group from collecting peripheral blood at all the stages of the study, and patient-related factors, such as genetic factors, nutritional condition and parallel use of other drugs.

Despite the existence of evidence concerning the role of these molecules in the course of leprosy in this study, we conclude that new investigations are necessary the evaluate the expression and the role of agonists and/or antagonists in either the proinflammatory or antiinflammatory effects, in order to confirm the presence of such molecules as disease-predicting markers and to serve as parameters for following-up the patient's immunological condition in future biological assays. A clearer understanding of the highly complex biological mechanisms involved will contribute to new therapeutic approaches.

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REFERENCES

1. Arango HG. Bioestatística: teórica e computacional. 2ª edição. Guanabara Koogan, Rio de Janeiro, 2005.
2. Araújo MG. Hanseníase no Brasil. Revista da Sociedade Brasileira de Medicina Tropical 36: 373-382, 2003.
3. Arend WP, Guthridge CJ. Biological role of interleukin 1 receptor antagonist isoforms. Annals of the Rheumatic Diseases 59: 60-64, 2000.
4. Barreto JA, Belone AFF, Fleury RN, Soares CT, Lauris JRP. Manifestações de padrão tuberculóide reacional na hanseníase dimorfa: estudo histoquímico e imuno-histoquímico comparativo, em biópsias cutâneas, entre reações tipo 1 ocorridas antes e durante a poliquimioterapia. Anais Brasileiros de Dermatologia 80: 68-74, 2005.
5. Cunha MGS. Episódos reacionais e relação com recidiva em doentes com hanseníase multibacilar tratados com diferentes esquemas terapêuticos. 2001. 163 f. Tese de Doutorado em Medicina, Faculdade de Medicina da Universidade de São Paulo - USP, Ribeirão Preto, 2001.
6. Ferrero-Miliani L, Nielsen OH, Andersen OS, Girardin SE. Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1² generation. Review. British Society for Immunology, Clinical and Experimental Immunology 147: 227–235, 2006.
7. Foss NT. Imunopatologia. *In:* Talhari S, Neves RG (eds) Hansenologia. 3ª edição. Gráfica Tropical, Manaus, cap.9, p.97-102, 1997.
8. Foss, N.T. Hanseníase: aspectos clínicos, imunológicos e terapêuticos. Anais Brasileiros de Dermatologia, Rio de Janeiro 74: 113-119, 1999.
9. Goulart IMB, Penna GO, Cunha G. Imunopatologia da Hanseníase: a complexidade dos mecanismos da resposta imune do hospedeiro ao *Mycobacterium leprae*. Revista da Sociedade Brasileira de Medicina Tropical 35: 365-375, 2002.
10. Harboe M. The immunology of leprosy. *In:* Hastings RC (ed) Leprosy. Churchill-livingstone, Edinburgh, cap. 4, p.53-87, 1985.
11. Hansson M, Ying G, Rosen H, Tapper H, Olsson I. Hematopoietic secretory granules as vehicles for the local delivery of cytokines and soluble cytokine receptors at sites of inflammation. European Cytokine Network 15: 167-176, 2004.
12. Kassan SS, Moutsopoulos HM. Manifestações clínicas e diagnóstico precoce da Síndrome de Sjögren. Archives of Internal Medicine 164: 1275-1284, 2004.
13. Kindler V, Sappino AP, Grau GE, Piguet PF, Vassalli P. The inducing role of tumour necrosis factor in the development of bactericidal granulomas during BCG infection. Cell V 56: 731-740, 1989.
14. Martelli CMT, Stefani MMA, Penna GO, Andrade ALSS. Brazilian endemisms and epidemics, challenges and prospects for scientific investigation: leprosy. Revista Brasileira de Epidemiologia 5: 273-285, 2002.
15. Mccullagh P, Nelder JA. An Outline of Generalized Linear Models. In, Generalized Linear Models. 2nd Edition, Chapman & Hall, London, p. 21-47, 1989.
16. Moubasher AEA, Kamel NA, Zedan H, Raheem DEA. Cytokines in leprosy, II. Effect of treatment on serum cytokines in leprosy. International Journal of Dermatology 37: 741-761, 1998.
17. Munk ME, Anding P, Schettini AP, Cunha MG, Kaufmann SH. Soluble Tumor Necrosis Factor Alpha Receptors in Sera from Leprosy Patients. Infection and Immunity 67: 423-425, 1999.
18. Noordeen SK. The epidemiology of leprosy. *In:* Hastings RC, Opromolla DVA (eds) Leprosy. Churchill-Livingstone, Edimburgh, cap.2, p.15-30, 1985.
19. Opromolla DVA. Noções de Hanseonologia. 2ª edição, Centro de Estudos Dr. Reynaldo Quagliato, Bauru, 2000.
20. Pardillo EF, Fajardo TT, Abalos RM, Scollard D, Gelber RH. Methods for the Classification of Leprosy for Treatment Purposes. Clinical Infectious Diseases 44: 1096-1099, 2007.
21. Pfaltzgraff RE, Bryceson A. Clinical Leprosy. *In:* Hastings RC (ed) Leprosy. Churchll Livingstone Inc. New York, p.134-176, 1985.
22. Pimentel MIF, Nery JAC, Borges E, Gonçalves RR, Sarno EN. O exame neurológico inicial na hanseníase multibacilar: correlação entre a presença de nervos afetados com incapacidades presentes no diagnóstico e com a ocorrência de neurites francas. Anais Brasileiros de Dermatologia 78: 561-568, 2003.
23. Rea TH, Modlin RL. *In:* Ftzpatrick, TB, Eisen AZ, Wolff K, Freedberg IL, Austen KF (eds) Tratado de Dermatologia, 5ª edição. Revinter Ltda, Rio de Janeiro, v2, 2005.
24. Rook GAW, Al Attiyah R. Cytokines and the Koch phenomenon.Tubercle 72: 13-20, 1991.
25. Sampaio EP, Sarno EN. Expression and cytokine secrection in the states of immune reactivation in leprosy. Brazilian Journal of Medical and Biological Research 31: 69-76, 1998.
26. Santos APT, Almeida GG, Martinez CJ, Rezende C. Imunopatologia da hanseníase: aspectos clínicos e laboratoriais. NewLab 73: 142-156, 2005.

27. Sarno EN, Grau GE, Vieira LMM, Nery J.A. Serum levels of tumor necrosis factor alpha and interleukin 1 during leprosy reactional stats. Clinical and experimental immunology 84: 103-108, 1991.

28. Scollard DM, Adams LB, Gillis TP, krahenbuhl JL, Truman RW, Williams DL. The continuing challenges of leprosy. Clinical Microbiology Reviews 19: 338-381, 2006.

29. Silva CL, Foss NT. Tumor necrosis factor in leprosy patients. Journal of Infectious Diseasis 159: 787-790, 1989.

30. Silva CL, Faccioli LH, Foss NT. Supression of human monocyte citokyne release by phenolic glicolip 1 of M. leprae. International Journal of Dermatology 61: 107-118, 1993.

31. Talhari S, Neves RG. Dermatologia Tropical: Hanseníase. 3ª edição, Editora Tropical, Manaus, v.167, p.2-131. 1997.

32. Talhari S, Neves RG, Penna GO, Oliveira MLV. Dermatologia Tropical: hanseníase. 4ª edição, Editora Tropical, Manaus, 2006.

33. Van Brakel WH. Peripheral neurophaty in leprosy and its consequences. Leprosy Review 71: 146-153, 2000.

34. Van Der Schouw YT, Verbeek ALM, Ruijs JHJ. ROC curves for the initial assessment of new diagnostic tests. Family Practice 9: 506-511, 1992.

35. Watson S, Bullck W, Nelson K, Schauf V, Gelber R, Jacobson R. Interleukin 1 production by Peripheral Blood Mononuclear Cells from leprosy patients. Infection and Imunnity 45: 787-789, 1994.