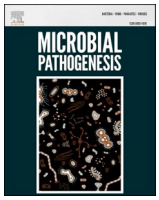
Microbial Pathogenesis 150 (2021) 104725

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath



CCL2 and IFN-γ serum levels as biomarkers for subclinical infection in household contacts of leprosy patients

Edson A. Queiroz [a], Nayara I. Medeiros [b], Rafael T. Mattos [b], Bruna F. Pinto [b], Ana Paula M. Carvalho [a], Walderez O. Dutra [b], Francisco C. Félix-Lana [a], Rodrigo Correa-Oliveira [a,c], Juliana A.S. Gomes [b,*]

[a] Escola de Enfermagem, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[b] Laboratório de Biologia das Interações Celulares, Departamento de Morfologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil
[c] Imunologia Celular e Molecular, Instituto René Rachou, Fundação Oswaldo Cruz - FIOCRUZ, Belo Horizonte, MG, Brazil

ARTICLE INFO

Keywords:
Leprosy
Household contact
Subclinical infection
Immunological biomarkers
CCL2
IFN-γ

ABSTRACT

Leprosy, also known as Hansen's disease, is a long-term infection by the bacteria *Mycobacterium leprae*, and actually still persists as a serious public health problem. The clinical parameters are used for diagnosis, however, some studies have indicated the selection of a set of biomarkers of subclinical infection, both serological and cellular, that allow the early diagnosis. Some cytokines and chemokines have been differentially expressed in index cases (paucibacillary and multibacillary patients) and household contacts (HHC), and may present a potential biomarker of *M. leprae* subclinical infection. Thus, the aim of this study was to analyze the variations in the profile of cytokines and chemokines, longitudinally, between index cases and their household contacts with a view to identifying possible biomarkers with differential expression, which may guide the early subclinical infection in household contacts. A longitudinal study was carried out between 2014 and 2015. The serum levels of the cytokines and chemokines were measured in all patient samples by CBA (*Cytometric Bead Array*). We observed a reduction of IL-4 and IL-17 expression of HHC group in the second evaluation (T1), as also a reduction of IL-17 in MB. We observed increased expression of IL-2 in PB patients as well. HHC, PB and MB showed a similar reduction profile of the chemokines CXCL8, CXCL9 and CXCL10 from T0 to T1. Interestingly, only serological levels of CCL2 are increased after a follow-up of HHC group, and this group, but not PB and MB patients, showed a significant association and a negative correlation between CCL2 and IFN-γ. The present study showed for the first time a similarity in the immunological scenario between HHC, PB and MB patients. In addition, this work highlights CCL2 chemokine in association with IFN-γ as possible biomarkers of subclinical infection of HHC, as also a parameter of early infection monitoring.

1. Introduction

Hansen's disease, also known as leprosy, is a contagious infectious disease caused by *Mycobacterium leprae*, which affects the skin and peripheral nerves, presenting varying degrees of clinical manifestation, from cases with few lesions (paucibacillary) to many lesions (multibacillary), with a long incubation period [1]. The disease still persists as a serious public health problem, with a global incidence of 210,671 cases in 2017. In Brazil, 26,875 new cases were registered, thus occupying the second overall position in the number of cases detected [2]. The diagnosis of leprosy is mainly based on the obligatory detection of clinical signs characteristic of the disease [1]. Several strategies are used to eliminate the disease, such as the improvement of the quality of health services, raising awareness and involving the community, vaccination with BCG (Bacillus Calmette-Guérin), and periodic clinical examination of household contacts in order to establish a diagnosis as early as possible [3]. However, the measures currently proposed have not been effective in eliminating the disease, since the incidence of leprosy and the diagnosis of new cases with grade 2 of physical disability continue with high rates and, the presence of new cases in children

* Corresponding author. Departamento de Morfologia, Instituto de Ciências Biológicas, UFMG Avenida Antônio Carlos, 6627. Pampulha, Belo Horizonte, Minas Gerais, 31270-010, Brazil.
E-mail address: juliana@icb.ufmg.br (J.A.S. Gomes).

https://doi.org/10.1016/j.micpath.2020.104725
Received 13 July 2020; Received in revised form 10 November 2020; Accepted 27 December 2020
Available online 2 January 2021

indicates the continuous transmission of the infection in the community [2]. This scenario is largely due to the continuity of the transmission chain, mainly from asymptomatic infected individuals who can spread the bacillus unknowingly [4].

In addition to the clinical parameters used in the diagnosis of leprosy, some studies have been working with a set of serological and cellular biomarkers of subclinical infection that allow the early diagnosis of the disease for possible implementation of chemoprophylaxis, preventing the chain of transmission of the infection and reducing the number of cases of the disease [5,6]. However, there are still no biomarkers with reliable sensitivity and specificity nor reliable quantitative or qualitative immunological parameters to detect subclinical infection by *M. leprae* and thus, predict the evolution of leprosy [7].

Cytokines and chemokines are important inflammatory mediators produced by different cell types that act on both the innate and adaptive immune response development [8,9]. Some studies have shown that these molecules are differently expressed in index cases and household contacts (HHC), and may present a potential biomarker of *M. leprae* subclinical infection [10–13]. On the other hand, the cytokine profile has also been measured to assess the effectiveness of the treatment for Hansen's disease [14]. Nevertheless, few studies have performed a longitudinal follow-up of index cases and household contacts and evaluated the profile of cytokine and chemokine as potential biomarkers of subclinical infection [15]. Thus, the aim of this study was to analyze the variations in the expression of cytokines and chemokines, longitudinally, between index cases and their household contacts with a view to identifying possible biomarkers with differential expression, which may guide the early subclinical infection in household contacts.

Table 1
Clinical, epidemiological, and demographic characteristics of the index cases (PB and MB) and household contacts.

Variable	HHC (n = 112)	PB (n = 18)	MB (n = 58)
Sex			
Male	47 (42%)	13 (72%)	19 (33%)
Female	65 (58%)	5 (28%)	39 (67%)
Age[a]			
Mean (±SD)	35 (±21)	50 (±16)	57 (±20)
Median	33	53	58
Minimum	7	18	12
Maximum	83	71	95
Presence of BCG Scar			
0	11 (10%)	5 (28%)	34 (59%)
1	46 (41%)	9 (50%)	20 (34%)
2	55 (49%)	4 (22%)	4 (7%)
Treatment time[b]			
Under treatment	NA	3 (20%)	11 (19%)
Treatment completed	NA	15 (80%)	47 (81%)
Characteristic of cohabitation			
Sleep in the same room	74 (66%)	NA	NA
Sleep in the same household	35 (31%)	NA	NA
Uninformed	3 (3%)	NA	NA
Length of time of contact with the case (in months)			
Mean (±SD)	243	NA	NA
Median	192	NA	NA
Relationship to contact with the index case			
No kinship or relationship	37 (33%)	NA	NA
Kinship or relationship	75 (67%)	NA	NA

Note: [a] Age at the time of the first interview.
[b] Treatment time at the time of the first interview/blood sample collection.
SD – Standard deviation; NA – Does not apply.

2. Patients, materials and methods

2.1. Ethical approval

This study was in accordance with the Helsinki Declaration and was approved and revised by the Research Ethics Committee of UFMG (Universidade Federal de Minas Gerais), protocol number #13639.

2.2. Study population

A longitudinal study was carried out between the years 2014 (Time 0 - T0) and 2015 (Time 1 - T1) in municipalities in the northeast of the state of Minas Gerais, Brazil, located in the Almenara microregion, composed of 14 municipalities, belonging to Cluster 6, considered a hyperendemic region for Hansen's disease [16]. The participating municipalities were: Almenara, Jacinto, Rubim, Jordânia, Palmópolis, Felisburgo. The study population consisted of index cases (IC) with paucibacillary (PB, n = 18) and multibacillary form (MB, n = 58) and household contacts (HHC, n = 112). All leprosy patients received the standard WHO-MDT regimen for leprosy: (1) Rifampicin and Dapsone for 6 months to TT/paucibacillary and (2) Rifampicin, Dapsone and Clofazimine for 12 months to LL/multibacillary. Fifteen of the 79 patients were being treated with standardized multidrug therapy in the first visit (Time 0 – T0) and the treatment was completed in it the second visit (Time 1 – T1). The rest of the cases had already completed their treatment. The volunteers were recruited through the National System of Grievances and Notifications (SINAN) database, along with secondary data related to the IC. Data collection was carried out through home visits, the use of a structured questionnaire, and collection of 20 mL blood, as per the agreement to participate set forth in the signed Informed Consent Form (ICF).

The dermatoneurological clinical examination was carried out on the household contacts to exclude clinical signs of the disease at the time of the home visit. No HHC showed such signs during the study. One inclusion criterion in this study was that the index cases had at least one household contact and another criterion was the permanence in this study of both. Women who were pregnant or suspected of being pregnant were excluded from the study.

2.3. Cytometric bead array (CBA)

The serum levels of the cytokines TNF, IL-6, IFN-γ, IL-2, IL-17A, IL-4 and IL-10 and the chemokines CXCL8 (IL-8), CCL2 (MCP-1 - monocyte chemoattractant protein-1), CXCL9 (MIG - monokine induced by interferon-γ) CXCL10 (IP-10 - interferon-γ-induced protein-10) were measured in all patient samples. The serum was centrifuged at 400 g for 40 min at 18 °C, aliquoted and then stored at −80 °C until the day of the experiment. Serum cytokines and chemokines were quantified using the CBA system (Becton Dickinson, BD-USA) in accordance with Medeiros and Gomes [17]. Data acquisition was performed by FACsCanto flow cytometer (BD-USA) and analyzed in the BD FCAP Array software, where values were expressed in pg/mL for both analytes.

2.4. Data analysis

Statistical analyses were carried out using the GraphPad Prism 6.0 software package (San Diego, CA, USA). The Shapiro-Wilk test was used to test data normality. To identify the outlier values was used ROUT (Robust Regression and Outlier removal) 1% of aggressiveness from GraphPad. Statistical comparisons were performed using the non-parametric two-tailed Mann-Whitney test for each variable. Statistical comparisons were performed using the paired *t*-test or Wilcoxon for parametric and non-parametric distribution, respectively. The

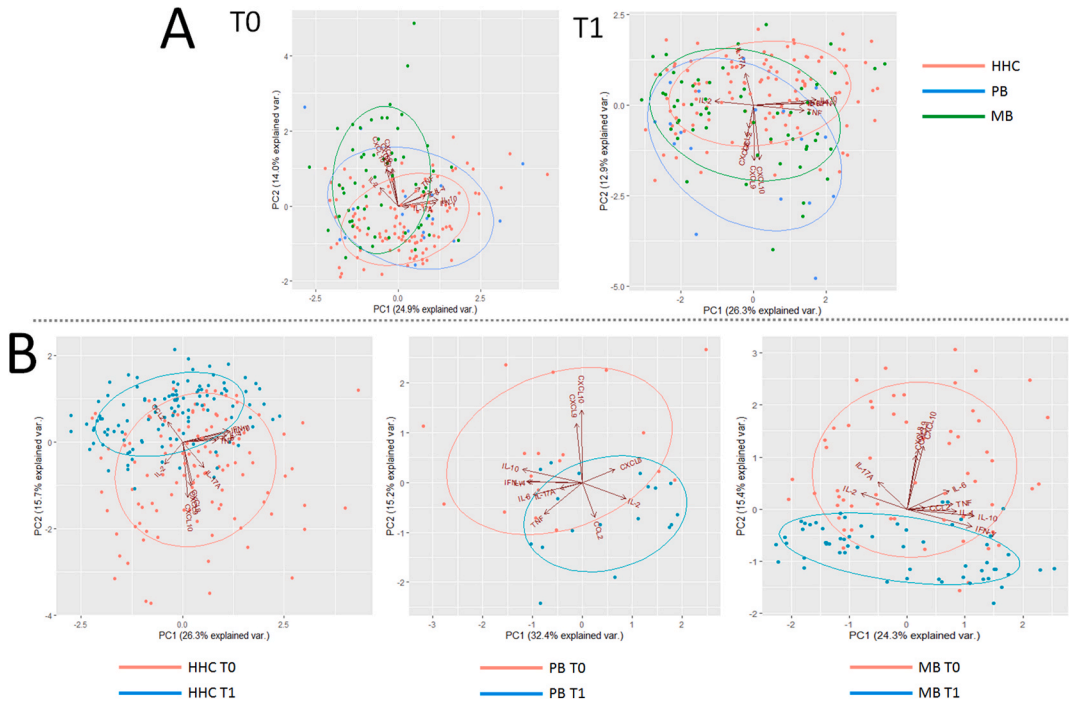


Fig. 1. Pattern of cytokine and chemokines expression becomes more homogeneous one years after first visit by principal component analysis (PCA). (A) PCA between HHC ($n = 112$), PB ($n = 18$) and MB ($n = 58$) in T0 (time 0) and T1 (time 1). Salmon indicate HHC ($n = 112$), blue PB ($n = 18$) and green MB ($n = 58$). **(B)** Salmon line indicate T0 and blue line indicate T1 for HHC, PB and MB patients.

association between CCL2 and cytokines in PB, MB and HHC groups was determined from the linear regression, considering the coefficient of determination (R^2) for the quality of fit and the F test to measure the variance between pairs ($p < 0.05$). Correlation analysis was done using the Spearman's (ρ) coefficient contained in the JMP software version 5.0.

To show the overall immunological profile between the index cases and household contacts, we used the multidimensional scaling approach (MDS) and in unpaired samples to verify the similarity/dissimilarity between the two groups, in order to provide an overview of how similar or not one group is to the other. It is an interdependence technique that allows you to map distances between points in a spatial graphical representation. Generally considered as exploratory data analysis because it reduces large amounts of data in easy-to-view structures. The dots are arranged within that space, so that the distances between peer pairs have the strongest possible relationship of similarities between pairs of objects. In other words, the points that are together represent similar objects, while different objects are represented by points that are distant. The Euclidean distances and their correlation coefficients were calculated from correlation matrices as pre-processing for the MDS using the cmdscale in the R Software database, version 3.4.2. Principal component analysis (PCA) also made for to show the overall immunological profile. This is a technique for reducing the dimensionality of such datasets, increasing interpretability but at the same time minimizing information loss. This analyze was mad in the R Software database, version 3.4.2.

The defined confidence interval was 95% and significant statistical differences were considered when $p < 0.05$.

3. Results

3.1. Clinical and demographic description of the population

The study population was composed of index cases, PB and MB treated and in treatment (n = 79) and household contacts (HHC) (n = 112), all accompanied during two years in a row (Table 1). The PB group presented an age of 50 ± 16 years (average ± standard deviation), of which 13 (72%) of the male and 5 (28%) of the female gender. The presence of BCG's vaccination scar was: 5 individuals (28%) without scarring and 13 (72%) with 1 or 2 scars. Three volunteers (20%) were still under treatment on their first visit. The MB group presented an age of 57 ± 20 years, of which 19 (33%) of the male and 39 (67%) of the female gender. The presence of BCG's vaccination scar was: 34 individuals (59%) without scarring, 20 (34%) with 1 and 4 (7%) with 2 scars. Eleven volunteers (19%) were still under treatment on their first visit (Table 1).

The HHC group presented 36 ± 21 years, of which 42% of the male and 58% of the female gender. The presence of BCG's vaccination scar was not found in 11 individuals (10%) and in 101 (90%) were found 1 or 2 scars. The cohabitation time with the IC presented an average of 243 months. In relation to cohabitation characteristics 74 (66%) slept in the same room and 35 (31%) slept in the same domicile. The relationship to contact with the index case was also evaluated and 75 (67%) had kinship and 37 (33%) had no degree of kinship (Table 1).

3.2. The cytokine and chemokine profile of HHC, PB and MB seems to be similar in a short time

To verify the immunological scenario from all the immunological parameters (TNF, IL-6, IFN-γ, IL-2, IL-17A, IL-4 and IL-10, CXCL8, CCL2, CXCL9 e CXCL10) of variations between PB, MB and your specific HHC in the T0 and T1 period we use principal component analysis (PCA). There was no segregation between the ellipses of all groups, both in T0 and T1, but there is clearly less variation in the data in HHC (in T0 and T1) and greater in PB (Fig. 1A). In Fig. 1B, the segregation of data in the follow-up of all groups is reasonably clear. The CCL2 chemokine vector stands out from the other cytokines in HHC and PB.

To measure the 'immunological space' (Fig. 2A) and the 'immunological distance' (Fig. 2B) from all the immunological parameters analyzed was used MDS approach. The data from the PB and MB was condensed in group IC (index case). In the 'immunological space', there was no separation of HHC and IC data (Fig. 2A), and the 'immunological

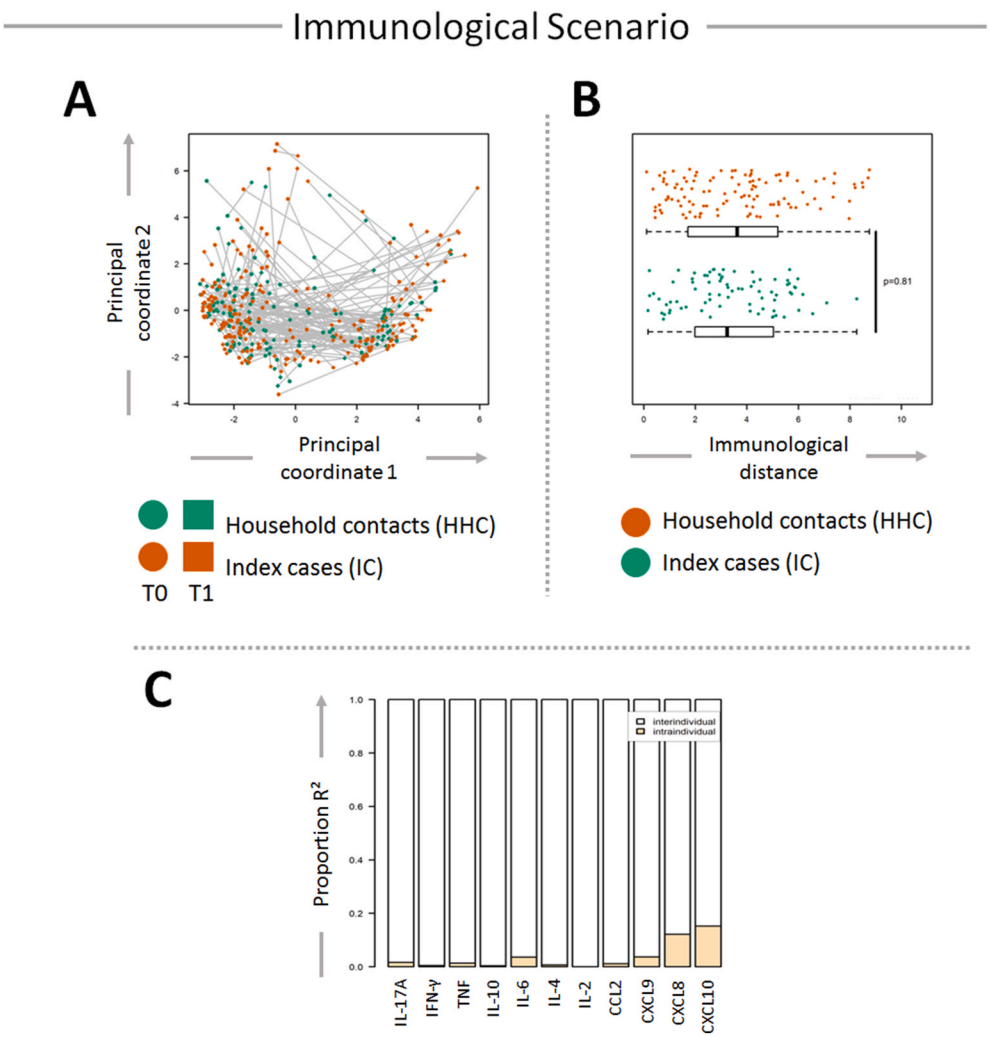


Fig. 2. The immunological scenario of variations between IC (index case) and your specific HHC. (**A**) MDS approach of time 0 (T0) and time 1 (T1) visits. Each individual's first and second visits are shown as circles and square, respectively; gray lines indicate the immunological distance (IC n = 79 and HHC n = 112 individuals; 382 visits in all). Green indicates household contacts (n = 112); orange indicates individuals with Hansen's disease (n = 79). (**B**) Quantification of the immunological distance between the first and second visits for household contacts (n = 112) versus individuals with Hansen's disease (n = 79). Two-tailed Mann-Whitney test was used to compare the immunological distances. Boxes and centerlines represent the interquartile range (IQR) and median, respectively. (**C**) The proportion of R^2 values from all volunteers attributable to either interindividual or intraindividual differences assessed for T0 and T1.

distance' between longitudinal samples from IC group was no greater than that of HHC individuals (p = 0,81) (Fig. 2B).

The variations in immune parameters analyzed between T0 and T1 samples of IC and HHC contributed very little to the observed total variation, with a median proportion of R^2 of 0.014 (range, 0.0001–0.15), while interindividual variation contributed much more for total variation, with a median proportion of R^2 of 0.986 (range, 0.847–0.999) (Fig. 2C).

3.3. *CCL2 has a distinct expression pattern when compared to other inflammatory mediators*

After assessing the immunological scenario of the PB, MB and HHC, we evaluated the intrinsic profile of inflammatory mediators of these groups before and after 2-years follow-up. Therefore, serum levels of cytokines (IFN-γ, TNF, IL-6, IL-2, IL-4, IL-10, and IL-17) and chemokines (CCL2, CXCL8, CXCL9, and CXCL10) were measured at two different times, T0 and T1. Our results showed a reduction of IFN-γ expression at T0 in MB group. The level of IL-2 in PB increased at follow-up. We also observed a reduction of IL-4 expression at T1 of the HHC, as well as a reduction of this cytokine at T0 of MB in comparison with the HHC group. In addition, HHC and MB had reduction of IL-17, in the follow-up at T1 (Fig. 3A) and less expression in PB when compared to HHC in T1.

When we evaluate the chemokines, it was possible to observe a reduction of CXCL8, CXCL9 and CXCL10 at T1 of both HHC and MB groups. In addition, our results showed reduction of CXCL10 expression in PB at T0 to T1. The MB group showed higher levels of CCL2, CXCL9 and CXCL10 compared to the HHC at T0 time. The MB group also had higher levels of CXCL9 at T1. The PB patients showed higher levels of CCL2, CXCL9 compared to the HHC at T0 and T1 time (Fig. 3B).

When comparing the differences between the expression of chemokines between PB and MB, we found that MB patients had higher expression of CCL2 and CXCL8 in T0 and lower expression of CXCL8 in T1 (Fig. 3B).

On the other hand, interestingly, only CCL2 expression showed higher at T1 of HHC and PB groups, as also a higher level of this cytokine at T0 of MB in comparison with HHC and PB was observed (Fig. 3B).

3.4. *CCL2 chemokine is negatively correlated with IFN-γ production only in HHC group*

Chemokines are chemotactic cytokines that control migration and placement patterns of immune system cells in tissues, participating in the organism's homeostasis [9]. Thereby, we analyzed the proportions of CCL2, CXCL8, CXCL9 and CXCL10 levels in serum of HHC, PB and MB groups at T0 and T1. It was possible to observe a reduction in the proportion of CXCL8, CXCL9 and CXCL10 chemokines from T0 to T1 in both HHC, PB and MB groups, with the exception of CXCL8 in PB. In contrast, serological CCL2 levels showed higher at T1 also all groups evaluated, and the HHC group demonstrated the largest proportion of CCL2 in the second evaluation (T1) (Fig. 4A).

Our next step was to evaluated through linear regression and

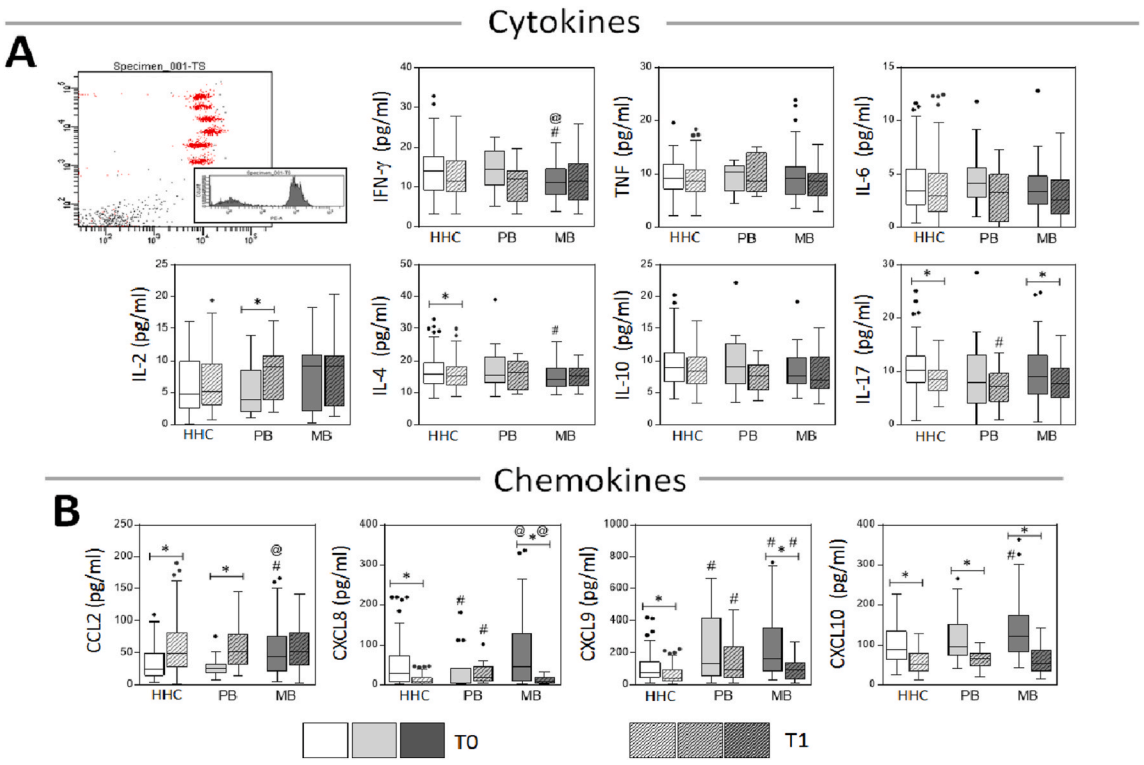


Fig. 3. Evaluation of serum levels of cytokines (A) and chemokines (B) in time 0 (T0) and time 1 (T1) were evaluated by cytometric bead array (CBA). Representative plot serum cytokine levels of one individual were shown at the up left. Serum concentration was shown in pg/mL. The bars highlight quartiles 1–3 and the whiskers highlight the lower and upper limits. The groups evaluated were the household contacts [HHC, n = 112 – white (T0) and cracked white (T1)] paucibacillary patients [PB, n = 18 - light gray (T0) and cracked light gray (T1)] and multibacillary patients [MB, n = 58 - dark grey (T0) and cracked dark grey (T1)]. Statistical differences ($p < 0.05$) between T0 and T1 were assessed according to the paired *t*-test or Wilcoxon for symmetric and non-symmetric distribution, respectively. Statistical differences between HHC, PB and MB were assessed according to the unpaired *t*-test or Mann-Whitney for symmetric and non-symmetric distribution, respectively.

correlation analysis the interaction between CCL2 chemokine and IFN-γ, TNF, IL-6, IL-2, IL-4, IL-10, and IL-17 cytokines at T1 of HHC, PB and MB groups. A significant association ($R^2 = 0.05/p = 0.02$), as also a negative correlation ($\rho = -0{,}25/p = 0.00$) between CCL2 and IFN-γ was found only in HHC group. Other significant differences were not observed (Fig. 4B).

4. Discussion

Currently, much of leprosy studies concentrate their efforts on the risk of illness of household contacts that may be developing clinical and immunological signs of subclinical infection [5,6,11,18]. It clearly showed less variation in the parameters analyzed in HHC both in T0 and in T1 compared to patients PB and MB, without however showing segregation of the ellipses, most likely due to the lower immune response generated by the lower exposure to the bacillus, unlike patients PB and MB. MB. Another clear observation is that in the follow-up, all groups evaluated showed less overall variation in the parameters analyzed. This is due to the effects of treatment and the resolving effects of the immune response to infection. In an attempt to verify the immunological scenario between HHC and cases (PB and MB grouped) the immunological similarity ("immunological distance and space") between the groups index cases and their home contacts remained in a short period of time. However, parameters with differential expression have been found and, bring us important information about intrinsic immunological characteristics of each group evaluated. We observed a reduction of IL-4 and IL-17 expression of the HHC group in the second evaluation (T1) as also a reduction of IL-17 of the MB group at T1. The cytokines IL-4 and IL-17 are essential to indicate the polarization of Th2 and Th17 helper T lymphocytes, respectively [19]. While Th2 lymphocyte is majority present in patients with the severe form of the disease (multibacillary) with IL-4 and IL-10 production and activation of T regulatory cells (T reg) [20,21], Th17 cells seems to have a protective function against *M. leprae* infection, potentiating IFN-γ production and inhibiting IL-10 production by Treg cells [22]. Moreover, IL-17 promotes several functions, among which it stimulates cellular immunity, improving resistance to leprosy [11], as well as participation in the effective formation of pulmonary granuloma in tuberculosis [23]. Thus, the reduction of these cytokines in healthy individuals after two years of contact with leprosy patients may be a balance between protective and regulatory immunity to prevent tissue damage. On the other hand, reduction of IL-17 after follow-up, as also of IL-10 at T1 when compared to T0 in the MB group suggests the loss of protection of these patients contributing to the emergence of intrinsic lesions to the disease.

At the follow-up, HHC, PB and MB show a similar reduction profile of the chemokines CXCL8, CXCL9 and CXCL10 from T0 to T1. These data indicate a reduction of the inflammatory process, largely due to the effects of treatment of PB and MB with a probable resolution of the infectious process by the immune system of HHC and patients. Interestingly, only serological levels of CCL2 are increased after follow-up of the HHC group. In addition, HHC group demonstrated the largest proportion of CCL2 in the second evaluation (T1) CCL2 is one of the main chemokines involved in the granuloma response and is the most important chemoattractant and activator for monocytes, recruiting CD4 and T lymphocytes [24]. The increase in CCL2 levels in HHC and PB, which are classically characterized by having a high cellular immune response [25], can be a protective factor against leprosy, since the decrease in the expression of chemokines could prevent the activation of host's chemotactic response and, therefore, *M. leprae* could escape destruction by the immune system, contributing to the establishment of

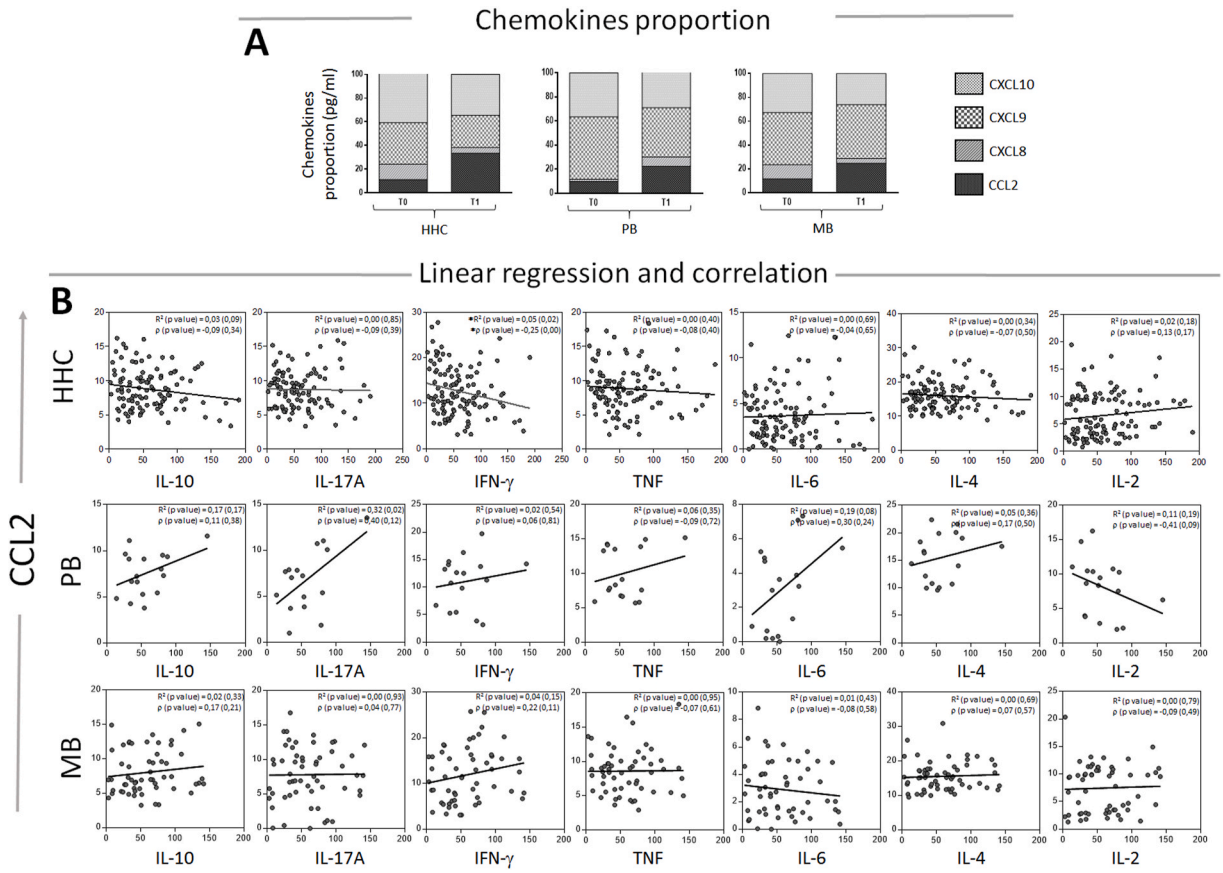


Fig. 4. The association between cytokine and chemokine in HHC, PB and MB. (A) Proportions of CCL2, CXCL8, CXCL9 and CXCL10 levels in serum of HHC, PB and MB groups at T0 and T1. **(B)** Linear regression and correlation analysis the interaction between CCL2 chemokine and IFN-γ, TNF, IL-6, IL-2, IL-4, IL-10 and IL-17 cytokines at T1 of HHC, PB and MB groups. Spearman's rank correlation coefficient was significant association was considered when $p < 0,05$.

intracellular infection and consequently the spread of the disease (Guerreiro, 2013). Kipnis et al. showed that CCL2 disrupted-gene in mice, submitted to low aerosol doses of *M. tuberculosis*, presented a small entry of macrophages in the lung and a transient increase in bacterial load [26]. Another evidence in favor of the CCL2 protective factor is that Hasan et al. (2006) showed a reduction in TNF-induced CCL2 expression in cell cultures of patients with the lepromatous form, which could contribute to the spread of the bacillus [27].

Another study indicated that TT/BT patients are more inclined toward a phenotype resembling that of tuberculosis patients with elevated CCL2 production [5]. Allied to this, IL-2 levels increased in the follow-up in BP. This interleukin, produced by the T lymphocyte, performs macrophage activation mediated by the production of IFN-γ, increasing the cellular response and contributing to protection against the bacillus.

After linear regression and correlation analysis, the HHC (but not PB an MB group) showed a significant association and a negative correlation between CCL2 and IFN-γ. These results suggest that although healthy individuals maintain direct contact with leprosy patients and thus develop an inflammatory process, mainly due to increased CCL2 chemokine, these individuals can reduce the inflammatory response by down-regulating IFN-γ production in serum.

The present study showed for the first time a similarity in the immunological scenario between HHC, PB and MB through the absence of segregation and 'immunological distance' evaluated by PCA and MDS approach, respectively. This find may be due to the effects of treatment and the resolution of the infectious process by HHC simultaneously. There is also the possibility of both groups have similar immune responses as a result of cohabitation, since most likely PB, MB and HHC share diet, living conditions, chronic infections, microbiome, well-being conditions, environmental pollutants, etc. [28,29], once the cohabitation between patients leprosy and HHC was an average of 243 months.

On the other hand, this work highlights that the balance/association of the expression of the chemokine CCL2 associated with IFN-γ in the follow-up, can serve as possible biomarkers of subclinical infection in HHC, considering the protective role of these molecules at appropriate levels. Thus, the periodic evaluation of the expression of these two molecules, in the serum of individuals living with leprosy patients, can be a new approach in monitoring the evolution of individuals and, thus, using early therapeutic approaches to prevent the development of the disease and the other people's infection.

Author summary

Leprosy, also known as Hansen's disease, is a long-term infection by the bacteria *Mycobacterium leprae*, and actually still persists as a serious public health problem. It is a chronic, infectious disease caused by *Mycobacterium leprae*, which affects the skin and peripheral nerves, presenting varying degrees of clinical manifestation. A number of strategies have been used to eliminate it, including periodic clinical examinations of household contacts intended to establish the earliest possible diagnosis. However, the incidence remains high. The aim of this study was to analyze the variations in the profile of important inflammatory mediators produced by different cell types that act on both the innate and adaptive immune response development (cytokines and chemokines in the present study), longitudinally, between leprosy patients and their household contacts with a view to identifying possible biomarkers with differential expression, which may guide the early subclinical infection in household contacts. We observed an overall reduction of cytokines and chemokines from Time 0 to Time 1, and an increase in the

expression of CCL2 in household contacts and paucibacillary patients at follow-up and finally a significant association and a negative correlation between CCL2 and IFN-γ. This work highlights CCL2 chemokine in association with IFN-γ as possible biomarkers of subclinical infection of HHC, as also a parameter of early infection monitoring.

Funding

JASG received sources of support provided by Conselho Nacional do Desenvolvimento Científico e Tecnológico (CNPq - #474796/2012) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG - #02419–15). EAQ, NIM, BFP, WOD, RCO and JASG received financial support from CNPq (PQ) fellowship program. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CRediT authorship contribution statement

Edson A. Queiroz: Formal analysis, Investigation, and writing and visualization of manuscript. **Nayara I. Medeiros:** Methodology, Investigation, Writing - review &a editing. **Rafael T. Mattos:** Investigation. **Bruna F. Pinto:** Writing - original draft. **Ana Paula M. Carvalho:** Investigation. **Walderez O. Dutra:** Supervision. **Francisco C. Félix-Lana:** Supervision, Resources. **Rodrigo Correa-Oliveira:** Conceptualization, Supervision, Project administration, Funding acquisition. **Juliana A.S. Gomes:** Conceptualization, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank the research group *Núcleo de Estudos e Pesquisas em Hanseníase* (NEPHANS), for its role in designing and participating in the data collection. We also thank the *Instituto de Ciências Biológicas* of UFMG and the *Instituto de Pesquisas René Rachou* (IRR)/ *Fundação Oswaldo Cruz*, for their support and assistance in the laboratory analyses. We give special thanks to the *Fundação de Amparo à Pesquisa de Minas Gerais* (FAPEMIG), the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) and PrInt-Fiocruz/ CAPES for their financial support.

References

[1] D.M. Scollard, et al., The continuing challenges of leprosy, Clin. Microbiol. Rev. 19 (2) (2006) 338–381.
[2] WHO, *Global leprosy update*, Accelerating reduction of disease burden, Wkly. Epidemiol. Rec. 93 (35) (2017) 445–456.
[3] World Health Organization Regional Office for South-East Asia New, D, Global strategy for further reducing the leprosy burden and sustaining leprosy control activities 2006-2010. Operational guidelines, Lepr. Rev. 77 (3) (2006) 1–50.
[4] S. Araujo, et al., Molecular evidence for the aerial route of infection of Mycobacterium leprae and the role of asymptomatic carriers in the persistence of leprosy, Clin. Infect. Dis. 63 (11) (2016) 1412–1420.
[5] A. Geluk, et al., New biomarkers with relevance to leprosy diagnosis applicable in areas hyperendemic for leprosy, J. Immunol. 188 (10) (2012) 4782–4791.
[6] I. Nath, C. Saini, V.L. Valluri, Immunology of leprosy and diagnostic challenges, Clin. Dermatol. 33 (1) (2015) 90–98.
[7] A. Geluk, Challenges in immunodiagnostic tests for leprosy, Expert Opin. Med. Diagn. 7 (3) (2013) 265–274.
[8] C.A. Dinarello, Historical insights into cytokines, Eur. J. Immunol. 37 (Suppl 1) (2007) S34–S45.
[9] J.W. Griffith, C.L. Sokol, A.D. Luster, Chemokines and chemokine receptors: positioning cells for host defense and immunity, Annu. Rev. Immunol. 32 (2014) 659–702.
[10] Z. Hasan, et al., Leprosy patients with lepromatous disease have an up-regulated IL-8 response that is unlinked to TNF-alpha responses, Int. J. Lepr. Other Mycobact. Dis. 72 (1) (2004) 35–44.
[11] F.B. de Almeida-Neto, et al., TH17 cells, interleukin-17 and interferon-gamma in patients and households contacts of leprosy with multibacillary and paucibacillary forms before and after the start of chemotherapy treatment, J. Eur. Acad. Dermatol. Venereol. 29 (7) (2015) 1354–1361.
[12] V.A. Mendonca, et al., Differentiation of patients with leprosy from non-infected individuals by the chemokine eotaxin/CCL11, Am. J. Trop. Med. Hyg. 77 (3) (2007) 547–550.
[13] R.D. Costa, et al., Evaluation of the expression of interleukin 1 beta (IL-1beta) and interleukin 1 receptor antagonist (IL-1Ra) in leprosy patients, Rev. Soc. Bras. Med. Trop. 41 (Suppl 2) (2008) 99–103.
[14] F. Cassirer-Costa, et al., Cytokines as biomarkers to monitoring the impact of multidrug therapy in immune response of leprosy patients, Cytokine 97 (2017) 42–48.
[15] S. Khadge, et al., Longitudinal immune profiles in type 1 leprosy reactions in Bangladesh, Brazil, Ethiopia and Nepal, BMC Infect. Dis. 15 (2015) 477.
[16] BRASIL, *Saúde Brasil 2009: Uma Análise da Situação de Saúde e da Agenda Nacional e Internacional de Prioridades em Saúde*, Ministério da Saúde, Brasília, 2010.
[17] N.I. Medeiros, J.A.S. Gomes, Cytometric bead array (CBA) for measuring cytokine levels in chagas disease patients, Methods Mol. Biol. 1955 (2019) 309–314.
[18] N.K. Madan, K. Agarwal, R. Chander, Serum cytokine profile in leprosy and its correlation with clinico-histopathological profile, Lepr. Rev. 82 (4) (2011) 371–382.
[19] M. Abdallah, et al., Estimation of serum level of interleukin-17 and interleukin-4 in leprosy, towards more understanding of leprosy immunopathogenesis, Indian J. Dermatol. Venereol. Leprol. 79 (6) (2013) 772–776.
[20] C. Saini, V. Ramesh, I. Nath, CD4+ Th17 cells discriminate clinical types and constitute a third subset of non Th1, Non Th2 T cells in human leprosy, PLoS Neglected Trop. Dis. 7 (7) (2013) e2338.
[21] R.L. Modlin, Learning from leprosy: insights into contemporary immunology from an ancient disease, Skin Pharmacol. Appl. Skin Physiol. 15 (1) (2002) 1–6.
[22] A.B. Fonseca, et al., The influence of innate and adaptative immune responses on the differential clinical outcomes of leprosy, Infect Dis Poverty 6 (1) (2017) 5.
[23] Y. Okamoto Yoshida, et al., Essential role of IL-17A in the formation of a mycobacterial infection-induced granuloma in the lung, J. Immunol. 184 (8) (2010) 4414–4422.
[24] M.F. Medeiros, et al., CXCL10, MCP-1, and other immunologic markers involved in neural leprosy, Appl. Immunohistochem. Mol. Morphol. 23 (3) (2015) 220–229.
[25] I.M. Goulart, G.O. Penna, G. Cunha, [Immunopathology of leprosy: the complexity of the mechanisms of host immune response to Mycobacterium leprae], Rev. Soc. Bras. Med. Trop. 35 (4) (2002) 365–375.
[26] A. Kipnis, et al., Role of chemokine ligand 2 in the protective response to early murine pulmonary tuberculosis, Immunology 109 (4) (2003) 547–551.
[27] Z. Hasan, et al., Elevated serum CCL2 concomitant with a reduced mycobacterium-induced response leads to disease dissemination in leprosy, Scand. J. Immunol. 63 (3) (2006) 241–247.
[28] P. Brodin, et al., Variation in the human immune system is largely driven by non-heritable influences, Cell 160 (1–2) (2015) 37–47.
[29] E.J. Carr, et al., The cellular composition of the human immune system is shaped by age and cohabitation, Nat. Immunol. 17 (4) (2016) 461–468.