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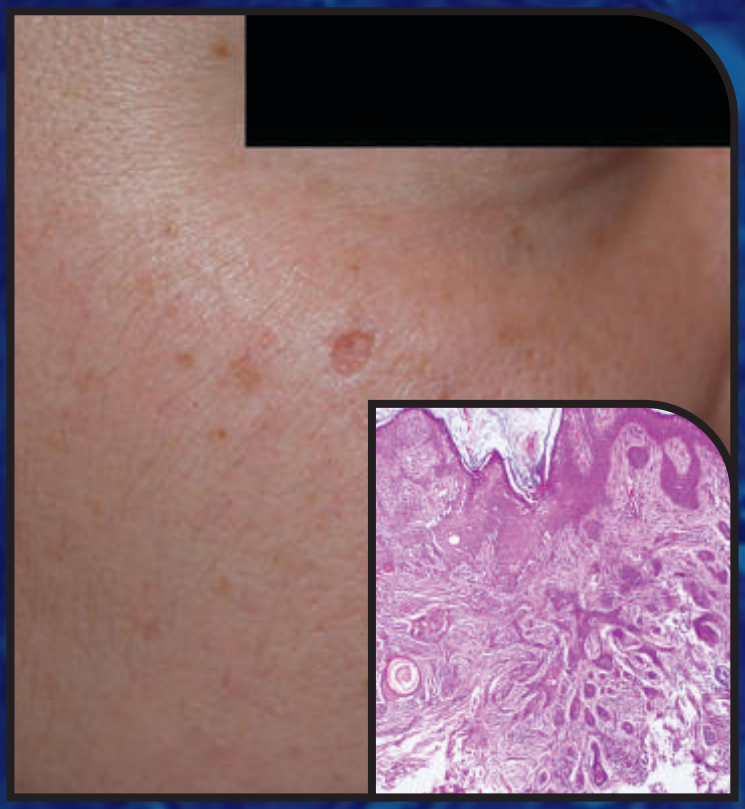

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Original Article

Estimation of serum level of interleukin-17 and interleukin-4 in leprosy, towards more understanding of leprosy immunopathogenesis

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ABSTRACT

Background: Combating *Mycobacterium leprae* is known to be via T-helper1 response. However, other T-helper effector cells; T-helper17 and T-helper2; play a role, particularly in the context of disease type. **Aims:** We aimed to evaluate serum levels of interleukin (IL)-17 (T-helper17 cytokine) and IL-4 (T-helper2 cytokine) in untreated patients with different types of leprosy, compared to controls. **Methods:** Using enzyme-linked immunosorbent assay, serum IL-17 and IL-4 levels were estimated in 43 leprotic patients and 43 controls. Patients were divided into six groups; tuberculoid, borderline cases, lepromatous, erythema nodosum leprosum (ENL), type 1 reactional leprosy, and pure neural leprosy. Patients were also categorized according to bacillary load and the presence or absence of reactions. **Results:** Serum IL-17 was significantly lower in cases (4-61.5 pg/mL; median 19), compared to controls (26-55 pg/mL; median 36) ($P < 0.001$), and was significantly lower in each type of leprosy compared to controls, with the lowest level in lepromatous leprosy (4-61.5 pg/mL; median 12.5). Significantly elevated serum IL-4 was found in patients (1.31-122.4 pg/mL; median 2.31) compared to controls (1.45-5.72 pg/mL; median 2.02) ($P = 0.008$), with the highest level among lepromatous leprosy patients (2-87.2 pg/mL; median 28.9), and the lowest in type 1 reactional leprosy (1.4-2.5 pg/mL; median 1.87) ($P = 0.006$). **Conclusion:** Defective secretion of IL-17 is related to disease acquisition as well as progression toward lepromatous pole in leprosy patients. The overproduction of IL-4 in patients with lepromatous leprosy may infer their liability to develop ENL. Nevertheless, the small number of the studied population is a limitation.

Key words: Interleukin-4, interleukin-17, leprosy

INTRODUCTION

Leprosy is a chronic granulomatous infection caused by the obligate intracellular organism; *Mycobacterium leprae* (*M. leprae*).[1] According to Ridley and Jopling,[2] leprosy is classified into tuberculoid (TT), borderline tuberculoid (BT), midborderline (BB), borderline lepromatous (BL), and lepromatous leprosy (LL). At one pole, TT leprosy is characterized by few bacilli (paucibacillary; PB) and vigorous cell-mediated immunity (CMI).[3] At the other pole, lies LL, with numerous bacilli (multibacillary; MB), and inefficient CMI.[4] TT is characterized by a predominance of CD4+ T cells and type-1 cytokines.[5-8] In contrast, LL is characterized by predominance of CD8+ T cells and type 2 cytokines.[7] Between those two polar forms, lie the borderline forms, liable to reactional leprosy (RL) type 1, with a predominantly type-1 cytokine profile.[9,10] Erythema nodosum leprosum (ENL), which manifests in BL and LL

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leprosy patients, is a more systemic reaction than the previous.[11,12] The aforementioned data demonstrate the immunopathological interactions between type 1 and type 2 cytokines[13,14] and activated macrophage products (monokines) in leprosy.[15] However, certain T cell subset were shown to produce cytokines that could not be classified according to the Th1-Th2 scheme. Interleukin (IL)-17 was among these cytokines[16] and T cells producing IL-17 were named Th17 cells.[17,18] Thus, we aimed in this study to evaluate serum levels of IL-17 and IL-4 in untreated leprosy patients, compared to healthy controls, to gain further insight into the role of these cytokines in the immunopathogenesis of leprosy.

METHODS

This study was conducted on 43 untreated leprotic patients attending at El Qal'aa Dermatology and Leprosy Hospital, Cairo, Egypt, and 43 healthy volunteers as a control group, after signing an informed consent, over a period of 10 months. The study was conducted according to the Declaration of Helsinki and was approved by the medical ethical committee of Ain Shams University, as well as National Research Institute. Patients were evaluated according to clinical examination, slit skin smear examination (SSS), and histopathological examination, and were divided into six groups: Group A: LL; Group B: TT; Group C: Borderline leprosy (BT, BB, and BL); Group D: RL type 1, Group E: ENL; and Group F: Pure neural leprosy (PNL). The patients were also categorized according to the presence or absence of RL into non-RL, including Groups A, B, C, and E, and RL, including Group D patients. In addition, 43 healthy age- and gender-matched subjects, with negative hepatitis C virus and hepatitis B virus serology and negative tuberculin test, were also included in the study, comprising the control group.

Blood samples were collected from both patients and controls, provided that all subjects were free of any other systemic disease. We excluded patients who started antileprotic treatment or who were taking any kind of immunomodulatory therapy likely to alter the results of the study, such as systemic corticosteroids. Blood was collected in sterile test tubes and centrifuged for 15 min at 50 g. Serum was separated and kept at −70°C until used for estimation of IL-4 and IL-17 by enzyme-linked immunosorbent assay (ELISA). IL-4 was quantitatively estimated by the RayBio® human ELISA kit (RayBio®, IL4-001, 2010, USA) according to the method described by Paul and Ohara (1987).[19] IL-17 was estimated by the RayBio® human ELISA kit (RayBio®, IL17-001, 2010, USA) according to the method described by Numasaki *et al*.[20]

Statistical analysis was done using SPSS (statistical program for social science) version 12. Mann-Whitney test for pair wise comparisons. Kruskal-Wallis test with Bonferroni adjustment was used for multiple comparisons. Spearman correlation was used to measure the correlation between the quantitative variables. A "*P*" < 0.05 was considered significant, while <0.001 was highly significant.

RESULTS

The current study included 43 leprotic patients; 16 females (37.2%) and 27 males (62.8%). Their ages ranged from 15 to 65 years [(mean ± standard deviation (SD) = 35.74 ± 11.23)]. Forty-three apparently healthy individuals served as controls; 16 females (37.2%) and 27 males (62.8%). Their age ranged from 15 to 60 years (mean ± SD = 36 ± 13.703). SSS was negative in 16 patients (37.2%), grouped as PB, while it was positive in 27 patients (62.8%); grouped as MB.[21] The patients subgroups were: Group A: 11 patients (25.6%) with LL, Group B: 6 patients (14%) with TT, Group C: 9 patients (20.9%) suffering from borderline leprosy (5 with BT, 3 with BB, and 1 with BL), Group D: 6 patients (14%) suffering from RL type 1, Group E: 6 patients with ENL (14%), and Group F: 5 patients (14%) suffering from PNL. Non-RL category included 31 patients (72.1%) from Groups A, B, C, and F, while RL included 12 patients (27.9%) comprising of group D and E patients.

Leprosy patients showed significantly lower IL-17 level (4-61.5 pg/mL; median of 19), compared to controls (26-55 pg/mL; median of 36) ($P < 0.001$). On comparing serum IL-17 level in patients with negative versus positive SSS, it ranged from 12.5 to 49 pg/mL (median = 20) in PB cases compared with 4-61.5 pg/mL (median = 19) in MB patients ($P = 0.989$). IL-17 was highest among TT (range: 14-49 pg/mL; median 21). The lowest level was detected in LL type (range: 4-61.5 pg/mL; median 12.5), with no statistically significant difference ($P = 0.223$). Nevertheless, statistically significant difference was found on comparing serum IL-17 levels in different types of leprosy with controls (median of 12.5 pg/mL in LL,

21 pg/mL in TT, 19 pg/mL in borderline, 19.5 pg/mL in RL Type 1, 20 pg/mL in ENL, and 14 pg/mL in PNL; compared to 36 pg/mL in controls) ($P = 0.007$, $P = 0.005$, $P = 0.018$, $P = 0.005$, $P < 0.001$, and $P < 0.001$, respectively). Yet, serum IL-17 levels were not statistically different in non-RL compared with RL patients (range: 4-61.5 pg/mL and median 17 vs. range: 12.5-50 and median 20, respectively) ($P = 0.671$).

Regarding serum IL-4, comparing patients and controls revealed highly significantly elevated serum IL-4 in patients compared to controls (range: 1.31-122.4 pg/mL and median 2.31 vs. range: 1.45-5.72 pg/mL and median 2.02, respectively) ($P = 0.008$). According to SSS, the level of IL-4 ranged from 1.87 to 7.26 pg/mL with a median of 2.24 in PB cases, while it ranged from 1.31 to 22.40 pg/mL with a median of 2.42 in MB cases ($P = 0.773$). IL-4 was highest among LL patients, ranging from 2 to 87.2 pg/mL (median = 28.9). The lowest level detected was in RL type 1, ranging from 1.4 to 2.5 pg/mL (median = 1.87). A statistically significant difference was found when comparing all groups (median of 28.9 pg/mL in LL, 2.67 pg/mL in TT, 1.94 pg/mL in borderline, 1.8 pg/mL in RL type 1, 2.4 pg/mL in ENL, and 2.1 pg/mL in PNL) ($P = 0.006$). Furthermore, serum level of IL-4 was statistically significantly higher in LL patients, compared to each of ENL, type 1 RL, borderline, and PNL groups ($P = 0.15$, 0.002, 0.01, and 0.009, respectively). Compared to controls, IL-4 level was statistically significantly higher in LL patients and TT patients (median of 28.9 and 2.67 pg/mL, respectively vs. 2 pg/mL for controls) ($P < 0.001$ and $P = 0.04$, respectively). Yet, serum IL-4 levels were not statistically different when comparing non-RL with RL (range: 1.31-87.2 pg/mL and median 2.31 pg/mL vs. range 1.43-3.5 pg/mL and median 2.1) ($P = 0.068$).

Negative correlation was detected between serum levels of both IL-17 and IL-4, but the results were not statistically significant ($r = -0.171$, $P = 1$) [Figure 1].

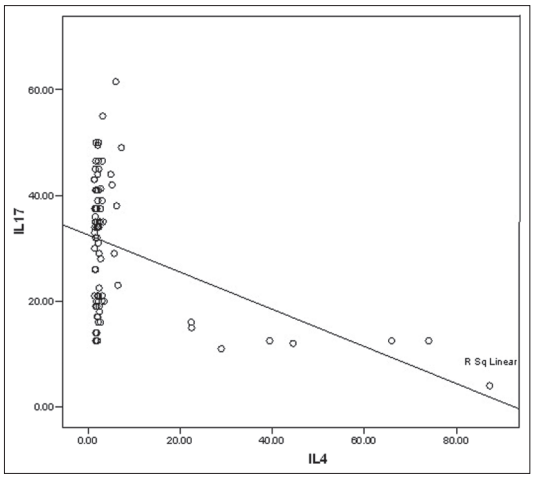

Figure 1: Correlation between interleukin-4 and interleukin-17

DISCUSSION

Hereby, it is worth mentioning that, to the best of our knowledge, Th17 and its cytokine profile were not studied in leprosy before. Th17 mechanism of induction and their effector function is nowadays the focus of important studies in immunology.[22] IL-17 was significantly lower in our cases compared to controls (the lowest in LL while the highest in TT). Studies on infection models described significant role of IL-17 level in mycobacterial infections; namely *M. tuberculosis*.[23] Susceptibility to pulmonary *M. avium-intracellulare* complex may be associated with biases in Th1/Th2/Th17 immunity.[24] Moreover, Th17 cells can provide interferon (IFN)-γ-independent protection against *M. tuberculosis*.[25] In accordance, in patients with tuberculosis disease, IL-17 was not detected in bronchoalveolar lavage fluid, which may be due to suppression by Th1 cytokines, including IFN-γ.[26] Thus, Th1 and Th17 responses cross-regulate each other during mycobacterial infection.[27] Another infection with similar pathology is leishmaniasis. The weak type 1 immune response observed in *L. braziliensis* infection may be mediated by poor innate immune response with impaired IL-17.[28] Therefore, we speculate that such an inherent deficiency can also contribute to the development of leprosy and even to disease progression toward the MB pole.

The hypothesis that the spectrum of leprosy reflects the balance between Th1 and Th2 populations is indeed exciting.[13] In TT, there is good evidence of predominant IL-2 and IFN-γ production, while LL patients have mainly cytokines of a Th2 type, including IL-4.[18] In accordance, we revealed significantly elevated IL-4 in patients, being highest among LL patients, and lowest in type 1 RL. Type 1 RL is associated with an overproduction of Th1-type cytokines.[12] Since Th1 and Th2 cells can cross-regulate one another; IFN-γ directly suppresses IL-4 secretion and Th2 polarization,[29] which is evident in type 1 RL. On the contrary, type 2 reactions occur in patients with poor CMI to *M. leprae*, abundant bacilli, and a strong polyclonal antibody response. In addition, increased IL-8 and IL-10, and sustained expression of IL-4 and IL-5; all cytokines associated with neutrophil chemotaxis and antibody

production were observed in ENL lesions.[30] However, there is also evidence of enhanced production of TNF-α and IL-6, and increased circulating IL-2 receptors in acute ENL episodes causing nerve destruction.[31] These findings can explain why serum IL-4 was not different in ENL patients compared to controls and other disease categories. On comparing serum IL-4 level in patients with PB versus MB leprosy, no significant difference was found. Moreover, no difference was detected, comparing RL to non-RL. In contrast, El Saadany *et al.*,[32] showed a significant difference in IL-4 among non-RL, type 1 reaction, and type 2 reaction, with a tendency to increased levels more in type 2 reaction. This data agrees with Verhagen *et al.*,[33] who stated that IL-4/IL-4 mRNA was produced predominantly from a BL patient. Since IL-4 inhibits CMI responses[34] and favors humoral immunity, IL-4 might contribute to high antibody levels and unrestricted replication of bacilli in such patients.[33] Studying this cytokine profile in both sera and tissues of larger leprosy population is recommended to clarify these points. Surprisingly, our TT patients had elevated IL-4 compared to controls. In contrast, Spellberg and Edwards[29] explained the absence of mRNA for IL-4 in BT or BB lesions, by the presence of Th1 cytokines IL-12 and IFN-γ. This discrepancy can be due to the difference between lesional and circulating cytokine profile, particularly toward the tuberculoid pole, the well-known of localized neurocutaneous disease, rather than being systemic disease, for further investigations. Negative correlation was detected between serum levels of both IL-17 and IL-4, but with no statistical significance. Since IL-17 is related to protective mechanisms against disease progression, while IL-4 could be related to disease progression, with Th2 activation; further, studies on larger number of patients can obviate a significant negative correlation between them.

Limitation

- The small number of patients prevented us from drawing solid conclusions.
- The absence of repeated measurements in the same individuals to evaluate whether treatment can attenuate this immune dysregulation is another limitation.

CONCLUSION

It seems that defective secretion of IL-17 has a role in leprosy progression. Targeting Th17 or IL-17 can be a future helpful approach to limit this endemic disabling disease. Since, *M. bovis* BCG-specific Th17 cells confer partial protection against *M. tuberculosis* infection,[27] the application of *M. leprae*-specific Th17 could be promising in the context of leprosy. On the contrary, the overproduction of IL-4 in MB leprosy patients may result in their liability to develop ENL. Approaches of IL-4 antagonism include soluble recombinant human IL-4 receptor; altrakincept[35] and a variant form of IL-4; pitrakinra.[36] To our knowledge, the use of such compounds in treatment of other IL-4-related disorders, such as BL and LL patients is not documented yet, for future trials.

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