Ben Dhifallah I, et al. Iran J Immunol. 2019; 16(4):299-310.
https://doi.org/10.22034/iji.2019.80281.

Iranian Journal of Immunology (IJI)
iji@sums.ac.ir

ORIGINAL ARTICLE

Decreased Level of IL-37 Correlates Negatively with Inflammatory Cytokines in Cerebrospinal Fluid of Patients with Neuro-Behcet's Disease

Imene Ben Dhifallah[1], Afshin Borhani-Haghighi[2], Agnes Hamzaoui[3,4], Kamel Hamzaoui[3,4*]

[1]Institut Pasteur de Tunis, Tunis, Tunisia, [2]Clinical Neurology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran, [3]Unit Research 12SP15 "Homeostasis and Molecular Dysfunction in the lung" Abderrahman Mami Hospital, Pavillon B, Ariana, [4]El Manar Tunis University, Faculty of Medicine of Tunis, Department of Immunology and Basic Sciences, Tunis, Tunisia

ABSTRACT

Background: Behçet's disease (BD) is a systemic inflammatory disease with a chronic, relapsing-remitting course of unknown etiology. Neuro-Behcet's disease (NBD) induce serious CNS complications and are known to be the main cause of long-term morbidity and mortality. IL-37 is a natural suppressor of innate inflammation which its role in NBD has not been fully understood. **Objective:** To determine the expression of IL-37 in cerebrospinal fluid (CSF) and its relationship with other inflammatory cytokines. **Methods:** Level of IL-37, IL-6, IL-17, IL-21, TSLP and TGF-β were measured in CSF of 22 patients with NBD and 12 non-inflammatory neurological disease (NIND) and 10 headache attributed to Behçet's disease (HaBD) by enzyme-linked immunosorbent assay (ELISA). In addition, IL-37 mRNA relative expression was detected by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). **Results:** CSF level and mRNA expression of IL-37 were elevated in NBD patients compared to those in NIND and HaBD patients. Levels of IL-6, IL-17, IL-21 and TSLP were found to be increased in NBD patients and were inversely associated with IL-37 level. Moreover, TGF-β level in CSF of NBD patients was positively correlated with IL-37 levels. IL-37 increased significantly after treatment and in remission group, but TGF-β was only increased in treatment group. **Conclusion:** IL-37 expression increased in NBD patients, and correlated with disease activity. Our data conclude that IL-37 could be a disease marker in NBD, however it requires further studies.

Received: 2019-01-19, Revised: 2019-07-25, Accepted: 2019-08-27.
Citation: Ben Dhifallah I, Borhani-Haghighi A, Hamzaoui A, Hamzaoui K. Decreased Level of IL-37 Correlates Negatively with Inflammatory Cytokines in Cerebrospinal Fluid of Patients with Neuro-Behcet's Disease. *Iran J Immunol.* 2019; 16(4):299-310. doi: 10.22034/iji.2019.80281.

Keywords: Interlukine-37, Inflammatory Cytokines, Neuro-Behçet's Disease

*Corresponding author: Dr. Kamel Hamzaoui, El Manar Tunis University, Faculty of Medicine of Tunis. Department of Immunology and Basic Sciences, Tunis, Tunisia, e-mail: kamel.hamzaoui@gmail.com

INTRODUCTION

Behçet's disease is an inflammatory disorder of unknown cause, characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis and skin lesions. Involvement of the central nervous system (CNS) and large vessels is less frequent, although it can be life-threatening. Neurological localisations in BD result in severe manifestations. Their frequency within BD patients ranges between 5% and 30% (1,2). CNS involvement is in the form of parenchymal NBD or non-parenchymal NBD (1,2). Innate and adaptive immunity are involved in NBD pathogenesis by releasing inflammatory cytokines in CSF (3-5). IL-6, IL-17 and IL-21 are responsible in part of immune dysregulation. The immunological imbalance favouring Th17 (IL-17) responses and suppressing regulatory T cells activity (Treg) driven by interleukin IL-21 secretion has been related with BD clinical activity. Immune cells in BD lesions produce high IL-6, IL-17, IL-9, IL-21 and IL-26 (6-8). Overall, recent and future advances in the knowledge of the pathogenesis of BD disease pave the way for the involvement of new mediators; we have recently reported the implication of IL-33 and TSLP in BD pathogenesis (9). TSLP and IL-33 are epithelium cytokines, TSLP acting through a dedicated receptor (TSLPR) (10). TSLP activates immature dendritic cells, leading to a high level of expression of CD80, CD86, and OX40L and chemokines production (11). Furthermore, TSLP enhances IL-33 expression (11). Current data points to the possible anti-inflammatory role of Interleukin-37 (IL-37) in BD, conducing to immune cell suppression (12,14).IL-37 is produced by various immune and resident cells (15). IL-37 forms a complex with (SMAD3), an important transcription factor in the TGF-β pathway, and is necessary for IL-37-driven responses. IL-37 suppresses innate and specific immune pathways (15,16), and is expressed in tissues to inhibit the eventual inflammatory response (17,18). TGF-β is a multifunctional cytokine that regulates development and repair of several cell types, including neurons (19). TGF-β favours IL-37 activity, enhancing its signals through ALK1 receptor complex binding, leading to activated pro-angiogenic responses (19). To distinguish the relationship between TGF-β and IL-37, we studied the expression of the latter in the CSF. The present research is the first to demonstrate that IL-37 is weakly expressed in NBD patients. We measured IL-37 protein and mRNA expression in the CSF of NBD patients, and its correlation with the inflammatory mediators (IL-6, IL-17, IL-21, TGF-β and TSLP) compared to age-matched patients suffering from NIND and HaBD.

MATERIALS AND METHODS

Ethical Approval. This investigation was performed at the Medicine University of Tunis (collaboration between the department of Immunology and Basic Science, Tunis; Tunisia and the Department of Neurology, Shiraz University of Medical Sciences, Shiraz, Iran). The working procedures conform to the ethical standards of the National Research Committee and the 1964 Helsinki Declaration. The work was approved by the ethical committee of the review board of Tunis Medicine University and the medical staff of the Institute of Neurology "Mongi Ben Hamida" (Tunis, Tunisia). Informed consent was obtained from all participants.

Patients. Twenty-two patients with NBD were enrolled from the Neurological institute. Twelve patients with NIND and 10 HaBD patients undergoing routine diagnostic

lumbar puncture acted as control diseases provided from the same neurological institute. NBD diagnosis was achieved according to the criteria established by the International Study Group for BD (ICBD criteria) (20). NBD patients' and CSF characteristics are described in Table 1. The treatment modalities consisted of azathioprine or methotrexate and corticosteroids as the first-line treatment.

Table 1. Clinical features of 22 Parenchymal Neuro-Behçet's Disease patients (NBD).

Clinical Features	
Gender	Males
Age (years, Min - Max))	42.7 (32 - 48)
Systemic involvements	n (%)
Oral ulcer	22 (100%)
Genital ulcer	22 (100%)
Pseudofolliculitis	12 (54.4%)
Positive pathergy test	16 (72.7%)
Erythemanodosum	10 (45.4)
Articular symptoms	12 (54.4%)
Ocular involvement	10 (45.4%)
Neurological involvement	18 (81.8)
Vascular involvement	14 63.6%)
Arterial aneurysms	13 (59%)
Arterial thrombosis	7 (31.8%)

For certain patients with high-risk, intravenous cyclophosphamide and corticosteroids are administered with or without immuno-suppressants. Anticoagulation was associated in case of cerebral vein thrombosis. Following 10 months of treatment, 10 patients lost most of their symptoms and were considered in the remission phase; IL-37 level was measured during both phases of the disease. The first control group was composed of 10 patients with HaBD. The mean age was (32.7 ± 6.8 years; range: 27-39). This type of headache should neither be accompanied by any focal or diffuse neurological signs or magnetic resonance imaging (MRI) findings nor fulfilling the HIS criteria for primary headaches (21). The second control group included12 patients suffering from NIND (8 patients with dementia and 4 patients with stroke; mean age: 42.6 ± 3.5 years; range: 26-42).

Biological Findings. C-reactive protein (CRP) was measured by ELISA (CRP high-sensitive ELISA, IBL International, Germany) according to the manufacturer's instructions. Erythrocyte sedimentation rate (ESR) was studied with the Westergren methods. These parameters were collected from patients' medical records at the time of admission. ESR mm/h: (32.27 ± 29.69); CRP mg/mL: (7.32 ± 3.58).

CSF-Mononuclear cells (CSF-MNC). CSF was stored at 4°C immediately after lumbar puncture. CSF was collected in pyrogen-free polypropylene tubes (Falcon, BD

Biosciences, Heidelberg, Germany). CSF immune cells were centrifuges at 2000 g during 10 minutes at 4°C) and were kept at -80°C until the time of the investigation. CSF-MNCs were kept in RPMI 1640 medium added to 10% foetal bovine serum and 1% antibiotics.

Enzyme-linked immunosorbent assay (ELISA). Human and mouse cytokine levels were measured by ELISA, according to the manufacturer's instructions as described previously (9). CSF IL-37 was measured following the manufacturer's instructions (human IL-37 ELISA, AdipoGen). The detected range of IL-37 was 0.016 to 1 ng/ml and sensitivity was 10 pg/ml. The protein levels of the IL-6, IL-17, IL-21 and TSLP in the CSF samples were measured according to the manufacturer's instructions. Each sample was tested in duplicate. The assay range of IL-6 (R&D Systems, Minneapolis, MN, USA) was 0.2 - 10 pg/ml and sensitivity was 0.11 pg/ml. The sensitivity for detecting IL-17 (Quantikine ELISA kits (eBioscience) was 15 pg/ml. Assay Range was 31.2 - 2,000 pg/ml. TGF-β1 (Quantikine ELISA Kit SB100B; R&D Systems, Inc., MN, USA). Sensitivity was 15.4 pg/ml and the assay range was 31.2-2,000 pg/ml. IL-21 (Thermo FisherScientific; Catalog # 88-8218-76) has an analytical sensitivity of 8 pg/ml and an assay range of 8-1,000 pg/ml. TSLP ELISA Kit (Creative-diagnostics ;CKERS-TSLP-156H) has a detection range: 7.8-500 pg/ml. The minimum detectable dose of TSLP was 2 pg/ml.

RNA extraction and RT-PCR. RNA samples were extracted by TRIzol reagent (Invitrogen), according to the manufacturer's instructions. cDNA was prepared using the iScriptc DNA Synthesis Kit (Bio-Rad). The primer sequences were as follows: for IL-37, sense: 5′-CTCCTGGGGGTCTCTAAAGG-3′and 5′-TACAATTGCAGGAGGTGCAG-3′(reverse); β-actin, 5′-CCTGACTGACTA CCTCATG AAG-3′ and anti -sense: 5′-GACGTA GCACAG CTTCTCCT TA-3'. Real-time PCR amplification reactions were prepared with the SYBR Green PCR Kit (Bio-Rad) and performed using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems). PCR products were verified by melting curve analysis. Relative expression levels of target genes were calculated by normalization to b-actin values using the $2^{-\Delta\Delta ct}$ method.

Statistical Analysis. Results were expressed as mean ± SD and were assessed by the Mann Whitney test. Correlations between different variables were analyzed using Spearman's rank correlation. p-value<0.05 was considered significant.

RESULTS

Expression of IL-37 in Neuro-Behcet's disease.

We compared IL-37 mRNA levels in CSF obtained from 22 NBD, 12 NIND and 10 HaBD patients measured by RT-PCR. IL-37 mRNA in NBD patients was significantly reduced compared to controls (NIND: p<0.0001; HaBD: p<0.0001) (Figure 1A). The CSF results of IL-37 level in NBD patients were in concordance with mRNA data (Figure 1B). Indeed, IL-37 level in active NBD patients was lower (33.36 ± 5.06 pg/ml) than NIND (40.91 ± 4.85 pg/ml; p=0.0002) and HaBD patients (40.20 ± 6.61 pg/ml; p=0.0031). No differences were observed between NIND and HaBD cases (p=0.772).

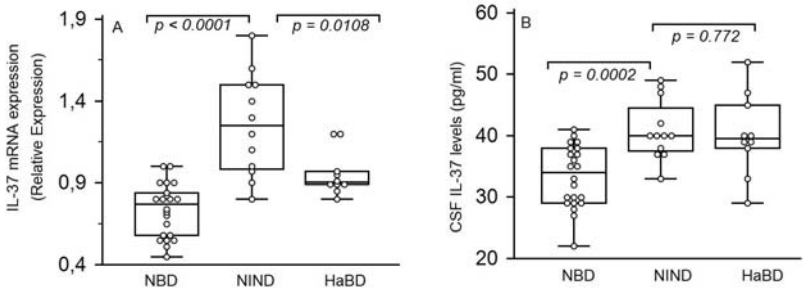

Figure 1. IL-37 expression Neuro-Behçet disease. [A]: Expression of interleukin-37 mRNA in cerebrospinal mononuclear cells (CSF-MNCs) from NBD compared to NIND and HaBD. Results are depicted as box plots, with median values, 25th and 75th quartile and the range of values. [B]: CSF levels of IL-37 and control diseases.

Correlation of IL-37 with IL-6, IL-17, IL-21, TSLP and TGF-β in CSF.

To identify perturbations in cytokines homeostasis, we analysed inflammatory cytokines IL-6, IL-17, IL-21 and TSLP in CSF obtained from NBD patients and their eventual correlations with IL-37. As shown in Table 2, NBD patients expressed higher levels of inflammatory cytokines than patients suffering from NIND and HaBD. IL-6 and IL-17 were expressed highly in NBD patients (IL-6: 49.27 ± 11.31 pg/ml; IL-17: 25.27 ± 8.40 pg/ml) compared to NIND (IL-6: 33.50 ± 4.20 pg/ml, p=0.0001; IL-17: 16.93 ± 5.17 pg/ml, p=0.0038) and HaBD (IL-6: 31.40 ± 5.81 pg/ml, p=0.0001; IL-17: 10.70 ± 2.31 pg/ml, p=0.0008) patients. IL-21, produced mainly by Th-17 cells exhibited also significant increase in NBD patients (12.52 ± 3.73 pg/ml) as compared to NIND patients (9.58 ± 1.6 pg/ml, p=0.0036) and HaBD patients (8.34 ± 1.88 pg/ml, p=0.0031).The concentration of TSLP remains slightly increased (45.36 ± 13.58 pg/ml) in the NBD compared to the NIND (33.58 ± 6.72 pg/ml; p=0.036) and HaBD (36.30 ± 4.27 pg/ml, p=0.044) patients. IL-37 was negatively correlated with the tested cytokines (IL-6, IL-17, IL-21 and TSLP) (Figure 2).

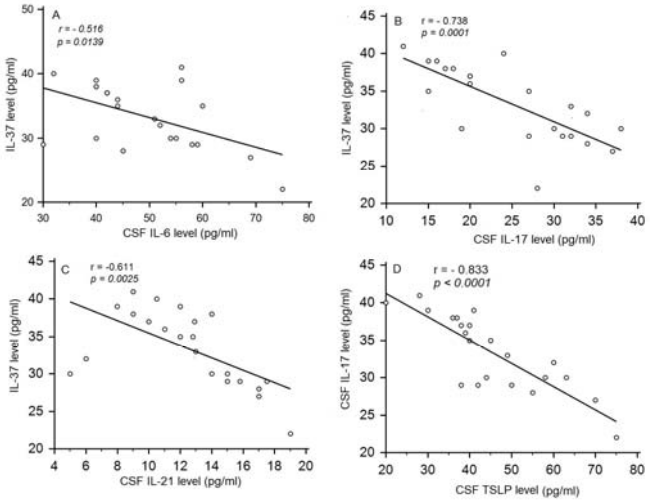

Figure 2. IL-6, IL-17, IL-21 and TSLP levels correlated to IL-37 expression in cerebrospinal fluid (CSF) from Neuro-BD patients. A negative correlation between the concentrations of CSF inflammatory cytokines and IL-37 were observed. The Coefficient of correlation (r) and p- values were determined using Spearman's correlation coefficient.

Table 2. Cytokines expression in the Cerebrospinal fluids from patients with Neuro-Behcet's disease compared to NIND and HaBD patients.

pg/ml	Neuro-BD	NIND	HaBD
IL-37	33.36 ± 5.06	40.91 ± 4.85 p=0.0002†	40.20 ± 6.61 p=0.0031††
IL-6	49.27 ± 11.31	33.50 ± 4.20 p=0.0001†	31.40 ± 5.81 p=0.0001††
IL-17	25.27 ± 8.40	16.93 + 5.17 p=0.0038†	10.70 ± 2.31 p=0.008††
IL-21	12.52 ± 3.73	9.58 ± 1.6 p=0.0126†	8.34 ± 1.88 p=0.031††
TSLP	45.36 ± 13.58	33.58 ± 6.72 p=0.0084	36.30 ± 4.27 p=0.049††
TGF-β	49.36 ± 9.53	86.66 ± 13.03 p=0.0001†	85.60 ± 10.0 p=0.0001††

(††): Statistical comparison between NBD and NIND patients and (††) between NBD and HaBD patients.

TGF-β level was significantly decreased in NBD (49.36 ± 9.53 pg/ml) patients compared to NIND (86.66 ± 13. 03 pg/ml; p=0.0001) and HaBD (85.60 ± 10.0 pg/ml; p=0.0001) (Figure 3A). In NBD-CSF the low levels of TGF-β1 were significantly correlated to the reduced levels of IL-37 (r=0.767; p<0.0001) (Figure 3B). Analysis of the eventual association between cytokine concentrations and clinical parameters revealed no correlation. This might suggest that this inflammatory process is independent of this or that clinical manifestation. It is the result of an immune manifestation that could lead to a phase of remission of the disease with disappearance of clinical manifestations. This could be demonstrated following the study of patients during the active and remission phases.

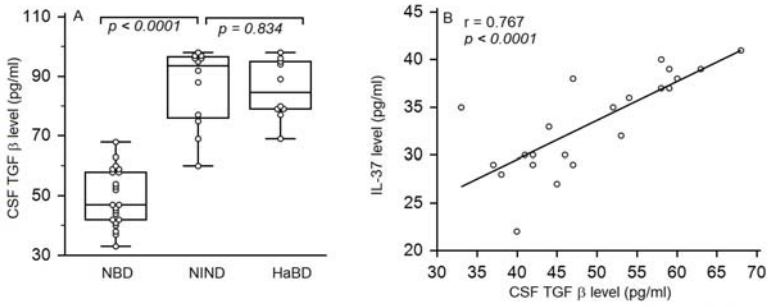

Figure 3. TGF-β expression in cerebrospinal fluid (CSF) levels in NBD and its correlation with IL-37. A positive correlation between the concentrations of CSF TGF-β and IL-37 was observed. The Coefficient of correlation and p-values were determined using Spearman's correlation coefficient.

Expression of IL-37 before and after treatment.

Ten active NBD patients were treated and considered in the remission stage as reported in method section. CSF was tested for IL-37, IL-6, IL-17 and TSLP levels. IL-37 levels

increased significantly (p=0.0002) (Figure 4A). Inflammatory mediators IL-6 and IL-17 were significantly reduced (p=0.001; p=0.0183) whereas no change was observed in TSLP (p=0.098) (Figure 4B, 4C, 4D).

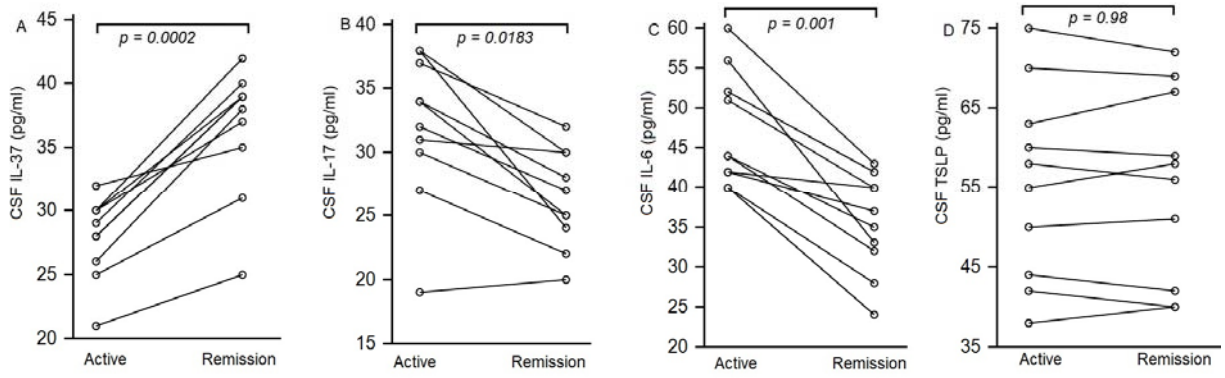

Figure 4. Cerebrospinal fluid IL-37 expression in Neuro-Behcet's disease before and after treatment. IL-37 and inflammatory cytokines were measured during active and remission stages. Treatment duration varies from 7 to 12 months. Significant differences were observed between the beginning and the end of the treatments. Statistical comparisons between the groups we remade using the Mann-Whitney *U* test.

The increased IL-37 level induces a relative regression of IL-6, IL-17 but does not seem to have any effect on the TSLP (Figure 4C; 4D, 4E). The increase in IL-37 following treatment demonstrates the effectiveness of treatment on the modulation of inflammatory mediators.

DISCUSSION

IL-37 was shown to be involved in immune disease as an anti-inflammatory cytokine and a regulator of both innate and acquired immune responses (15). Considering the inflammatory status in NBD, we found that both IL-37 levels and its mRNA expression in CSF were dramatically low in NBD patients compared to NIND and HaBD. A concomitant increase in CSF of IL-6, IL-17, IL-21 and TSLP levels was found in NBD patients. In contrast, TGF-β was significantly decreased in NBD. IL-37 protein was negatively associated with IL-6, IL-17, IL-21 and TSLP inflammatory cytokines. However, the attempt to find a possible correlation between inflammatory and suppressive cytokines and clinical parameters in NBD was vein. More importantly, we show for the first time, to our knowledge, that IL-37 increased markedly in remission stage compared to the active NBD stage. The recovery of the balance of homeostasis by suppression of IL-6, IL-17, IL-21 and TSLP is essentially conditioned by medical treatments inducing a rebalancing of the immune system with a regression of inflammatory mediators. In BD, recent works reported either a decrease or normal values of IL-37 levels in the peripheral circulation to those observed in healthy subjects (4,8,12-13,22-23), but until now, no data were reported about IL-37 levels in the central

nervous system on BD. Özgüçlü *et al.* (12) reported that IL-37 levels evaluated in Turkish patients were similar in BD and HC groups. They indicated that IL-37 level was positively associated with BD having mucocutaneous manifestations. Tan *et al.* (22) reported that IL-37 and IL-18RAP gene polymorphisms were associated with BD and there was a significant difference between BD cases and healthy controls for two SNPs (rs2058660 and rs3811047) in two genes (IL-18Rap and IL-37). In accordance with our results, Ye *et al.* (23) indicated that both IL-37 mRNA level and protein expression were significantly decreased in PBMCs from active BD patients compared to normal controls. They showed that the stimulation of dendritic cells (DCs) with rIL-37, inhibited remarkably Th17 and Th1 cell responses as compared to control DCs. Ye *et al.* concluded that the decreased IL-37 expression in active BD patients may trigger the production of pro-inflammatory cytokines in association with Th1 and Th17 cells. Through these different reports, notable differences are observed that could be due to differences in the ethnicity of studied populations. BD is related to more than one pathogenic pathway triggered by different environmental factors such as infectious agents in genetically predisposed subjects. Our present findings suggest the occurrence of a specific CSF inflammatory environment in NDB compared to disease controls. The inverse correlation observed between inflammatory cytokines and IL-37 probably indicated an intense immune dysregulation in the CNS, with the anti-inflammatory IL-37 tending to turn the corner to establish a balance. To the best of our knowledge, the monitoring of NBD disease activity is traditionally based exclusively on clinical observations and laboratory values, IL-37 could be considered as a supplement to NBD diagnosis. Therefore, our data suggest that the inflammatory response observed in the CSF could induce the upregulation of IL-37, thus exerting its immunosuppressive role. An interesting question that emerges from these findings is "What is the cellular source of IL-37 in the CNS". Extensive investigations are needed to detect whether cells in the nervous system could produce IL-37. However, considering the crucial role of T cell hypersensitivity to different types of antigens in BD pathogenesis, great number of $CD3^+CD4^+$ memory T cells accumulates in BD inflammatory sites and in CSF (24-25) and may be involved in the activation of the immune system during inflammation (26-27). Recent data indicates that IL-37 is predominantly detected in $CD3^+$ $CD4^+$T cells, from patients with Rheumatoid Arthritis (28). These data probably reflect the fact that in BD, CSF - activated immune cells, $CD3^+CD4^+$ T cells are the mostly like source of IL-37. Therefore, IL-37 - mediated anti-inflammatory effects could be mediated by $CD4^+$ T cells. The expression of inflammatory cytokines and IL-37 during the active and inactive phases of NBD was necessary to determine the expression of all these actors of immunity and to be able to show a recovery of homeostasis during the remission of the disease. The increased IL-6 level in CSF of NBD patients was reported by Hirohata *et al.* (29) suggesting that it may induce neurons apoptosis. IL-6 is very potent at inducing IL-17 secretion from memory $CD4^+$ T cells and driving Th17 cell differentiation (30,31). Th17 cells and their signature cytokine IL-17 are involved in the development, as well as the progression of NBD. The increased IL-17 expression in serum and in CSF from patients with BD was clearly demonstrated (32-33). IL-21 was implicated in neutrophils and monocytes chemotaxis to CSF through the blood brain barrier, in several autoimmune diseases (34). Of note, neutrophils and IL-21 play a pivotal role in BD inflammation (34-35). TSLP and at the same level IL-33 are epithelial cytokine, they have inflammatory role in BD (8,36-37). Recent data suggested an inflammatory role of IL-33 in NBD contributing to CNS activation of cellular immunity and

enhancing signalling processes that mediate neurons and oligodendrocytes injury (36). TSLP plays an important role in T-lymphocyte maturation and activation. This cytokine is positioned between $CD4^+$ T lymphocytes and resident cells as a response to in situ aggression against the latter. Kitic *et al.* reported that TSLP was produced in the spinal cord by astrocytes and in the CNS by choroid plexus epithelial cells (38). The precise role of TSLP in NBD or in human CNS is yet to be known, and only very few studies have been published assessing a definitive role to TSLP-Th17-driven autoimmune diseases (38). In our results, the fact that TSLP is inversely correlated with IL-37 suggests that TSLP production by the resident cells was as a result of the inflammation. We suggest that TSLP may have an inflammatory role given its high secretion as good as IL-17, IL-6 and IL-21. Current information suggests that the expression of several inflammatory cytokines, essential to experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis pathways, is impaired when TSLP is missing (39). We reported a significant positive correlation between TGF-β and IL-37. Masuda *et al.* (40) reported values of TGF-β in normal controls which were similar to values observed in the present data regarding in HaBD and NIND patients. TGF-β acts both as a growth-inhibiting and growth-promoting factor, the former aspect being often more important than the latter (41). The major role of TGF-β in the immune system is to control the severe aspects of inflammation, T and B cell differentiation and activation (42). TGF-β could affect differentiation of $CD4^+$T cells; hence produce more IL-17 (43-44). During BD activity, our results indicated that the low IL-37 level has little suppressive effect on IL-17, IL-6, IL-21 and TSLP secretion. It is likely that the immunosuppressive effect of IL-37 on inflammatory state is weak. Actually, recent evidence suggests that IL-37 significantly suppressed Ag-specific adaptive immunity through the induction of tolerogenic DCs and the promotion of IL-10 expression on DCs (42). Similarly, the presence of IL-37 markedly inhibited DC function and Th17 cell differentiation - related cytokine production (e.g. IL-6 and IL-21) (14). Notably, DCs are the main APCs mediating Th17 responses by secreting anti-inflammatory or proinflammatory cytokines, such as IL-6, TGF-β, and IL-10 (43). These results indicate that IL-37 is capable of regulating DC function and phenotype directly or indirectly and thereby, inhibiting inflammatory responses. In BD a malfunction of dendritic cells (DC) was suggested (34,44). DCs stimulated with rIL-37 showed a decreased expression of IL-6, IL-1β and TNF-α, and a higher production of IL-27. rIL-37 significantly inhibited the production of ROS by DCs and reduced the activation of ERK1/2, JNK and P38 MAPK in DCs. rIL-37-treated DCs remarkably inhibited Th17 and Th1 cell responses as compared to control DCs (23). The increased IL-37 production during the BD remission phase limited the intensity of inflammation by suppressing the IL-17, IL-6, IL-21 and TSLP cytokines response. Although the molecular mechanism of IL-37 in autoimmune/inflammatory disease remains unknown, recent studies showed that IL-37 acts as an extracellular cytokine by binding IL-18R and IL-1R8 for its anti-inflammatory properties (45). In addition, IL-37 fails to inhibit IL-17–triggering cytokine (IL-1-β and IL-6) production and the MAPK signalling pathway in DCs from IL-1R8–deficient mice (45). A recent study showed that IL-17 expression was dramatically enhanced in IL-1R8–deficient mice (46). Nevertheless, further investigations and manipulations of IL-37 signalling may improve therapeutic options for BD as well as in autoimmune/inflammatory diseases. The IL-37 anti-inflammatory activity was not as powerful and effective as reported by works concerning other pathologies. In the BD remission stage following treatment IL-37 increased

significantly, attenuating the in vivo production of IL-6 and IL-17 yet not significantly influencing TSLP production. However, certain questions remain unanswered: why is IL-37 not acting on the decline of the TSLP? Are higher doses of IL-37 required for immunomodulation to occur in resident cells? Immune pathways in BD currently appear particularly complex probably due to ignorance of its aetiology. Different treatment modalities in NBD patients may affect the levels of pro- and anti-inflammatory mediators. However, steroids, azathioprine and corticosteroids have no specific impact on particular cytokines. Steroids inhibit all transcription of all cytokines through blockade of activator protein-1 and NF-κβ (47). Azathioprine and methotrexate are anti-metabolites with no specific action against a particular cytokine (48). This study has certain limitations. First, the immune mechanism between IL-37 and the various inflammatory cytokines is limited, new studies on the molecule and the mechanism of IL-37 are needed. Secondly, the small amount of CSF and the scarcity of immune cells do not allow further investigation. Probably the cloning of lymphocytes would be a first solution to complete molecular investigations. Finally, it is important to determine if glial or neuronal cells could secrete IL-37. In summary, as far as the authors of the research are concerned, the current data represent the first characterization of IL-37 in NBD, highlighting its correlations with IL-6, IL-17, IL-21 and TSLP. However, more functional work is needed to identify the possible mechanisms of IL-37 as a suppressor of inflammatory cytokines against resident cells, and to better define its mode of action with central nervous system cells. In addition, our work may prompt further investigation of IL-37 in the central nervous system of other diseases.

ACKNOWLEDGEMENTS

This work was supported by « Ministère de la recherché Scientifique ». We thank all patients who allowed us to do this work. We also thank the technical staff of the Immunology Laboratory at Charles Nicolle Hospital of Tunis.

REFERENCES

1. Farahangiz S, Sarhadi S, Safari A, Borhani-Haghighi A. Magnetic resonance imaging findings and outcome of neuro-Behcet's disease: the predictive factors. Int J Rheum Dis. 2012; 15:e142-9.
2. Borhani-Haghighi A, Safari A. Proposing an algorithm for treatment of different manifestations of neuro-Behcet's disease. Clin Rheumatol. 2010; 29:683- 6.
3. Borhani-Haghighi A, Ittehadi H, Nikseresht AR, Rahmati J, Poorjahromi SG, et al. CSF levels of cytokines in neuro-Behçet's disease. Clin Neurol Neurosurg. 2009; 111:507-10.
4. Kaabachi W, Bouali E, Berraïes A, Dhifallh IB, Hamdi B, et al. Interleukin-26 is over expressed in Behçet's disease and enhances Th17 related-cytokines. Immunol let. 2017; 190:177-84.
5. Bonacini M, Soriano A, Zerbini A, Calò E, et al. Higher Frequencies of Lymphocytes Expressing the Natural Killer Group 2D Receptor in Patients with Behçet Disease. Front Immunol. 2018; 9:2157.
6. Pineton de Chambrun M, Wechsler B, Geri G, Cacoub P, Saadoun D. New insights into the pathogenesis of Behcet's disease. Autoimmu Rev. 2012; 11:687-698.
7. Kaabachi W, Mnasria K, Hamdi B, Khalfallah I, Ammar J, Hamzaoui K, et al. Th 9 cells in Behçet disease: Possible involvement of IL-9 in pulmonary manifestations. Immunol Lett. 2019; 211:3-12.

8. Kacem O, Kaabachi W, Dhifallah IB, Hamzaoui A, Hamzaoui K. Elevated expression of TSLP and IL-33 in Behçet's disease skin lesions: IL-37 alleviate inflammatory effect of TSLP. Clin Immunol. 2018; 192:14-9.
9. Quentmeier H, Drexler HG, Fleckenstein D, Zaborski M, et al. Cloning of human thymic stromal lymphopoietin (TSLP) and signalling mechanisms leading to proliferation. Leukemia. 2001; 15:1286.
10. Ito T, Wang YH, Duramad O, Hori T, et al. TSLP-activated dendritic cells induce an inflammatory T helper type 2 cell response through OX40 ligand. J Exp Med. 2005; 202:1213-23.
11. Kim BS, Siracusa MC, Saenz SA, Noti M, et al. TSLP elicits IL-33–independentinnatelymphoidcellresponses to promote skin inflammation. Sci Transl Med. 2013; 5:170ra16.
12. Özgüçlü S, Duman T, Ateş FSÖ, Küçükşahin O, et al. Serum interleukin-37 level and interleukin-37 gene polymorphism in patients with Behçet disease. Clin Rheumatol. 2019; 38:495-502.
13. Bouali E, Kaabachi W, Hamzaoui A, Hamzaoui K. Interleukin-37 expression is decreased in Behçet's disease and isassociated with inflammation. Immunol Lett. 2015; 167:87-94.
14. Boraschi D, Lucchesi D, Hainzl S, Leitner M, Maier E, et al. IL-37: a new anti-inflammatory cytokine of the IL-1 family. Eur Cytokine NetW. 2011; 22:127-47.
15. Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, et al. IL-37 is a fundamental inhibitor of innate immunity. Nat Immunol. 2010; 11:1014-22.
16. Takimoto T, Wakabayashi Y, Sekiya T, Inoue N, Morita R, et al. Smad2 and Smad3 are redundantly essential for the TGF-β–mediated regulation of regulatory T plasticity and Th1 development. J Immunol. 2010; 185:842-55.
17. Akdis M, Burgler S, Crameri R, Eiwegger T, Fujita H, et al. Interleukins, from 1 to 37, and interferon-γ: receptors, functions, and roles in diseases. J Allergy Clin Immunol. 2011; 127: 701-721. e70.
18. Böttner M, Krieglstein K, Unsicker K. The transforming growth factor-βs: structure, signaling, and roles in nervous system development and functions. J Neurochem. 2000; 75:2227-40.
19. Zhao M, Hu Y, Jin J, Yu Y, Zhang S, et al. Interleukin 37 promotes angiogenesis through TGF-β signalling. Sci Rep. 2017; 7:6113.
20. International Study Group for Behçet's Disease. Disease, International study group for Behcet's disease. Lancet. 1990; 335:1078-80.
21. Olesen J, Steiner T. The International classification of headache disorders, 2nd edn (ICDH-II), BMJ Publishing Group Ltd. 2004.
22. Tan H, Deng B, Yu H, Yang Y, Ding L, Zhang Q, et al. Genetic analysis of innate immunity in Behcet's disease identifies an association with IL-37 and IL-18RAP. Sci Rep. 2016; 6:35802.
23. Ye Z, Wang C, Kijlstra A, Zhou X, Yang P. A possible role for interleukin 37 in the pathogenesis of Behcet's disease. Curr Mol Med. 2014; 14:535-42.
24. Hamzaoui K, Borhani-Haghighi A, Ghorbel IB, Houman H. RORC and Foxp3 axis in cerebrospinal fluid of patients with neuro-Behçet's disease. J Neuroimmunol. 2011; 233:249-53.
25. Belghith M, Bahrini K, Kchaou M, Maghrebi O, Belal S, Barbouche MR. Cerebrospinal fluid IL-10 as an early stage discriminative marker between multiple sclerosis and neuro-Behçet disease. Cytokine. 2018; 108:160-167.
26. Lucherini OM, Lopalco G, Cantarini L, Emmi G, Lopalco A, Venerito V, et al. Critical regulation of Th17 cell differentiation by serum amyloid-A signalling in Behcet's disease. Immunol Lett. 2018; 201:38-44.
27. Deniz R, Tulunay-Virlan A, TureOzdemir F, Unal AU, Ozen G, et al. Th17-Inducing Conditions Lead to *in vitro* Activation of Both Th17 and Th1 Responses in Behcet's Disease. Immunol Invest. 2017; 46:518-525.
28. LianYe L, Jiang B, Deng J, Du J, Xiong W, Guan Y, et al. IL-37 Alleviates Rheumatoid Arthritis by Suppressing IL-17 and IL-17-Triggering Cytokine Production and Limiting Th17 Cell Proliferation. J Immunol. 2015; 194:5110-9.
29. Hirohata S. Histopathology of central nervous system lesions in Behcet's disease. J Neurol Sci. 2008; 267: 41-7.
30. Hu Y, Shen F, Crellin NK, Ouyang W. The IL-17 pathway as a major therapeutic target in autoimmune diseases. Ann N Y Acad Sci. 2011; 1217:60-76.

31. Miossec P, Kolls JK. Targeting IL-17 and TH17 cells in chronic inflammation. Nat Rev Drug Discov. 2012; 11:763-76.
32. Leccese P, Alpsoy E. Behçet's Disease: An Overview of Etiopathogenesis. Front Immunol. 2019; 10:1067.
33. Saruhan-Direskeneli G, Yentür SP, Akman-Demir G, Işik N, Serdaroğlu P. Cytokines and chemokines in neuro-Behçet's disease compared to multiple sclerosis and other neurological diseases. J Neuroimmunol. 2003; 145:127-34.
34. Türe-Özdemir F, Tulunay A, Elbasi MO, Tatli I, Maurer AM, Mumcu G, et al. Pro-inflammatory cytokine and caspase-1 responses to pattern recognition receptor activation of neutrophils and dendritic cells in Behcet's disease. Rheumatology (Oxford). 2013; 52:800-5.
35. Geri G, Terrier B, Rosenzwajg M, Wechsler B, Touzot M, et al. Critical role of IL-21 in modulating TH17 and regulatory T cells in Behcet disease. J Allergy Clin Immunol. 2011; 128:655-64.
36. Hamzaoui K, Borhani-Haghighi A, Kaabachi W, Hamzaoui A. Increased interleukin 33 in patients with neuro-Behcet's disease: correlation with MCP-1 and IP-10 chemokines. Cell Mol Immunol. 2014; 11:613-6.
37. Koca SS, Kara M, Deniz F, Ozgen M, Demir CF, et al. Serum IL-33 level and IL-33 gene polymorphisms in Behçet's disease. Rheumatol Int. 2015; 35:471-7.
38. Kitic M, Wimmer I, Adzemovic M, Kögl N, Rudel A, et al. Thymic stromal lymphopoietin is expressed in the intact central nervous system and upregulated in the myelin-degenerative central nervous system. Glia. 2014; 62:1066-74.
39. Eckhardt J, Döbbeler M, König C, Kuczera K, et al. Thymic stromal lymphopoietin deficiency attenuates experimental autoimmune encephalomyelitis. Clin Exp Immunol. 2015; 181:51-64.
40. Masuda T, Itoh J, Koide T, Tomidokoro Y, et al. Transforming growth factor-β1 in the cerebrospinal fluid of patients with distinct neurodegenerative diseases. J Clin Neuro sci. 2017; 35:47-49.
41. Ghoreschi K, Laurence A, Yang XP, Tato CM, et al. Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. Nature. 2010; 467:967-71.
42. Luo Y, Cai X, Liu S, Wang S, Nold-Petry CA, et al. Suppression of antigen-specific adaptive immunity by IL-37 via induction of tolerogenic dendritic cells. Proc Natl Acad Sci U S A. 2014; 111:15178-83.
43. Guermonprez P, Valladeau J, Zitvogel L, Théry C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. Annu Rev Immunol. 2002; 20:621-667.
44. Pay S, Simsek I, Erdem H, Pekel A, Musabak U, Sengul A, et al. Dendritic cell subsets and type I interferon system in Behçet's disease: does functional abnormality in plasmacytoid dendritic cells contribute to Th1 polarization? Clin Exp Rheumatol. 2007; 25:S34-40.
45. Li S, Neff CP, Barber K, Hong J, Luo Y, et al. Extracellular forms of IL-37 inhibit innate inflammation *in vitro* and *in vivo* but require the IL-1 family decoy receptor IL-1R8. Proc Natl Acad Sci U S A. 2015; 112:2497-502.
46. Bozza S, Zelante T, Moretti S, Bonifazi P, DeLuca A, et al. Lack of Toll IL-1R8 exacerbates Th17 cell responses in fungal infection. J Immunol. 2008; 180:4022-4031.
47. Wiseman AC. Immunosuppressive Medications. Clin J Am Soc Nephrol. 2016; 11:332-43.
48. Jabs DA. Immunosuppression for the Uveitides. Ophthalmology. 2018; 125:193-202.