In vivo and in vitro IL-18 production during uveitis associated with Behçet disease: Effect of glucocorticoid therapy

Production de l’IL-18 in vivo et ex vivo pendant l’uvéite associée à la maladie de Behçet : effet de la corticothérapie

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Summary Uveitis represents one of the major diagnostic criteria in Behçet’s disease. It is most prevalent in the countries of the Mediterranean area, including Algeria, and along the Silk Road. Clinical features include oral and genital ulcers, ocular and skin lesions, as well as central nervous system, joint, vascular, gastrointestinal, or pulmonary manifestations. Many studies have reported that Th1 immune responses are involved in the physiopathology. We have previously studied the production of IL-12 and IFN-γ, cytokine markers in the Th1 pathway involved in Behçet’s disease. In our study, we investigate in vivo and in vitro IL-18 production in Algerian patients with Behçet’s disease with ocular manifestations in various stages of the disease.

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Introduction

Behçet’s disease (BD) is a chronic systemic inflammatory disorder characterized by a course of remissions and exacerbations of unpredictable frequency and duration. This disease is considered as a vasculitis, affecting vessels of different types, sizes, and localizations [1]. It is most prevalent in the countries of the Mediterranean area, including Algeria, and along the Silk Route. The clinical features include oral and genital ulcers, ocular and skin lesions, as well as central nervous system, joint, vascular, gastrointestinal, or pulmonary manifestations. The treatment comprised systemic corticosteroids and immunosuppressants [2].

Despite the presence of regional and ethnical variability in the clinical expression, uveitis is one of the major disease manifestations [3]. Although it is not a life threatening manifestation, it has a heavy socio-economic impact. In fact, blindness frequency, due to relapsing ocular inflammation, occurs in about 70% of the patients, even if intensive immunosuppressive therapy is provided [4].

The cause and pathogenesis of BD are still unknown, but the onset of the disease is believed to be triggered by external environmental factors (Herpes simplex virus, streptococcal infection) in subjects with particular genetic susceptibilities (association with HLA-B51, MICA genes). Immunoregulatory abnormalities suggesting an autoimmune context have also been proposed as pathogenic mechanisms [5]. Moreover, several reports suggested that autoimmunity mechanisms might play a crucial role. The presence of immunoreactivity against vascular and ocular structures especially retinal proteins such as visual arrestin (S-Ag) and interphotoreceptor retinoid-binding protein (IRBP) had been reported [6,7]. Furthermore, some clinical and pathological findings suggests an auto-inflammatory component [8]. Indeed, neutrophil hyperactivity has also been suggested as one of the main pathogenic mechanisms in BD [9]. In addition, over-expression of Pattern recognition receptors (PRRs) as TLR2 and TLR 6 has been shown on patient’s immune cells [10,11].

Cytokines play a key role in Behçet physiopathology. In the last few years, an increasing number of data have reported the involvement of chemokines (IL-8, MCP-1), pro-inflammatory cytokines (IL-1β, TNF-α) and immune-modulatory cytokines (Th differentiation driven markers) in disease pathogenicity and/or activity [12]. In the same way, the production of tumor necrosis factor (TNF-α), IL-6, and IL-8 has been found to be elevated [13]. Furthermore, spontaneous or induced over-expression of pro-inflammatory and Th1 type cytokines has been shown in various cellular sources. Pro-inflammatory cytokines seem to be responsible for the enhanced inflammatory response in BD [14,15]. In our previous studies, we reported the overproduction of either Th1 (IL-12, IFN-γ), Th2 (IL-4) or Treg (IL-10) during the different stages of the disease [16–19]. In addition, we observed an inflammatory markers production (IL-8 and nitric oxide) increase in during Behçet uveitis. In addition, the elevation of IL-8 and nitric oxide was correlated with disease activity. Interestingly, patients’ treatment with corticoids during active stages resulted in a significant diminution in the production of these molecules in relation with the clinical disease stages [20].

IL-18 is a member of IL-1β family. It is constitutively expressed as a pro-form of 22 kDa and its 18 kDa active form is released after the action of caspase-1. IL-18 has multiple roles potentially affecting both innate and adaptive immune responses since IL-18Rα is expressed in almost all immune cells and some non-immune cells. Activated monocytes and macrophages are the main cellular source of IL-18. However, a robust IL-18 production can be obtained following stimulation with microbial products that cause the activation of...
cells and NK cells when combined with IL-2 and induces the secretion of IL-4, IL-5 and IL-10 in vivo in mouse models of disease [21].

Increased amounts of circulating IL-18 has been reported during Behçet disease with or without ocular manifestations. However, we noted heterogeneity in the values found by the different studies [22—24]. In our current study, our goal is to investigate in vivo and ex vivo IL-18 production in BD Algerian patients with ocular manifestation. We measured its production during Behçet uveitis at the two clinical stages of the disease. Furthermore, we examined the glucocorticoids effect on IL-18 production during the acute phase of the disease knowing that glucocorticotherapy is the usual therapy of this disease.

**Patients and methods**

**Patients and samples**

We included 26 Algerian patients fulfilling the diagnostic criteria of the International Study Group for BD. Patients were followed in ophthalmology and internal medicine departments of Mustapha Bacha and Bab El Oued CHU. The mean (SD) duration of the disease was 7.5 ± 6.7 years (range 1.5—15), and the patients’ ages ranged from 21 to 48 years (mean 34). Disease activity was assessed by clinical monitoring for BD (Table 1). We excluded patients with active stage for all the extraocular lesions. We also excluded all patients under immunosuppressant therapy.

To analyse the relationship with disease activity, we followed the IL-18 levels in 16 cases of patients hospitalised during acute phase and treated with pulse corticosteroids therapy (methylprednisolone) from 120 mg/day to 500 mg/day depending on clinical findings. Patients with retinal attack (as macular edema) were treated with the highest doses (500 mg/day), while patients with wilder presentation (as anterior uveitis) were treated with the lowest doses.

Sex- and age-matched healthy volunteers were included as normal controls (n = 17). All subjects in this study provided informed consent, and the study was approved by the local Ethics Committee (ATRSS: Algerian national agency for research in health sciences).

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<th>Table 1 Patients and control clinical and demographic data.</th>
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**Cells separation and culture**

Peripheral blood mononuclear cells (PBMCs) were obtained by separating heparinized venous blood on Histopaque (Sigma-Aldrich) as previously described [20]. Viable mononuclear cells that excluded Trypan blue were counted (viability always > 98%) and then diluted in RPMI medium with FBS at 10% to a concentration of 10^6 cells/ml. All experiments were performed in multi-well cell culture plates, and cultures were set up in triplicate. Cultures of PBMCs were stimulated by the addition of either phytohaemagglutinin (PHA, 10 μg/ml) or IL-18 (100 pg/ml). Cultures were incubated for 24 h in 5% CO2 at 37°C. After incubation, supernatants were stored for cytokine assay at −70°C.

**Cytokines’ measurement**

IL-18 concentrations were measured in patients’ sera and culture supernatants by ELISA (IL-18: MBL, MA). IFN-γ was measured in culture supernatants using an enzyme immunoassay (IFN-γ: Life Technologies, USA) according to the procedure suggested by the manufacturers. A standard curve was used to quantify the cytokine’s levels. The lowest sensitivity was <12.5 pg/ml for IL-18 and <0.03 U/l/ml for IFN-γ. Data were expressed as mean ± SEM.

**Statistical analyses**

The Mann–Whitney U test was used for comparisons between groups. The results were considered significant when the P-value was less than 0.05.

**Results**

**In vivo IL-18 production**

In our study, we observed an increased in vivo IL-18 production during Behçet uveitis in Algerian patients. In addition, we noticed that patients in active stage had higher IL-18 concentrations than those in the inactive stage (P < 0.01). During inactive stage, IL-18 levels were similar to those of control subjects (P = 0.34) (Fig. 1). We observed a significant reduction in IL-18 levels during patients’ treatment (P < 0.05) in correlation with clinical improvement (Fig. 2).

**Ex vivo IFN-γ modulation by IL-18**

In order to state IL-18 involvement in Th1/Th2 balance during Behçet uveitis, we cultured patients’ PBMCs in...
presence of IL-18 (100 pg/ml) during 24 h in 5% CO2 at 37 °C. We observed that culture treatment with IL-18 induced a significant increase in IFNγ production by cultured cells from patients and control subjects (P < 0.05). However, IL-18 effect was significantly higher in the patients’ cell culture supernatants by comparison to control subjects’ cell cultures (P < 0.01) (Fig. 3).

Ex vivo IL-18 production

In addition to the in vivo measurement, we also studied ex vivo IL-18 production by peripheral blood mononuclear cells (PBMCs). Our results showed that patients with Behçet uveitis had a significant increased IL-18 production in comparison to control subjects (P < 0.05). In fact, in our study, we found that PBMCs from patients with ocular involvement produced significantly higher amounts of IL-18 in comparison to control subjects. Furthermore, we observed that the ex vivo production profile was similar to the previously observed in vivo profile with significant differences between patients in active stage and control subjects (P < 0.01). T cell stimulation with PHA increased significantly IL-18 in all categories but patients’ cell responses were significantly higher than those of control subjects (P < 0.05) (Fig. 4). Interestingly, the treatment inhibitory effect is still observed ex vivo even without adding glucocorticoids to the medium. However, it could not inhibit the effect of PHA (Fig. 5).
These cytokine manifestations similar several lar has in studies, our study in regulating the inflammatory response in vivo. In fact, this cytokine has been detected in inflammatory fluids/tissues of several autoimmune diseases such as the cerebrospinal fluids of multiple sclerosis, the synovium of rheumatoid arthritis patients, the inflamed mucosa of Crohn’s disease, psoriatic plaques and the atheroma of atherosclerotic patients [32]. Increased amounts of circulating IL-18 has been reported during Behçet disease with or without ocular manifestations [22–24]. Our results are in accordance with those observations in our cohort of Algerian patients and comfort the possible pathological role of IL-18 during BD. Our study is the first to focus on the study of IL-18 production during ocular involvement in Behçet disease. In fact, we observed significant differences between patients depending on disease activity. Musabak et al. in 2006 showed a correlation between disease score and IL-18 levels. However, he did not show significant difference between patients in active and inactive stages [24]. In addition, our patients in active stage did not indicate differences with controls and our results were less heterogeneous (Fig. 1). This could be related to the study of a single manifestation leading to a more controlled monitoring of the disease expression and activity. This could also be due to ethnic or regional specificities. Our data are in the line with the results reported in Behçet Tunisian patients with ocular and other manifestations [22,25]. The authors observed an increase in IL-18 in active stages suggesting an association between IL-18 production and BD activity [22]. The previously observed high production of IL-18 during inactive stage could be related to the initiation, yet clinically silent, of the immune processes leading to the active stage or to the presence of a background Th1 immune activation during inactive stage [24].

In our study, the observed high increase of IL-18 production in all tested patients during active stage suggest that IL-18 could act in early stage of inflammatory response in Uveitis during Behçet Disease and its presence is closely related to IFN-γ production (Figs. 1 and 3). In order to confirm the possible systemic presence of IL-18 before the apparition of the clinical symptoms, it will be interesting to monitor the cytokine production during relapse periods and so, if it would be possible to predict the instalment of ocular inflammation.

As cited before, IL-18 increase is associated to different clinical manifestation of BD. However, its implication in the genesis of ocular inflammation is not clear. In fact, while IL-18 has been found in aqueous humor of patients with other uveitis [33], there is no available data on the local IL-18 production during Behçet uveitis. In addition, as BD diagnosis is based on the presence of at least one more additional clinical manifestation, it will be interesting to compare the observed systemic results with those of patients presenting a non Behçet uveitis in order to confirm the implication of systemic IL-18 production in Behçet uveitis physiopathology.

In autoimmune diseases, autoimmunity is characterized by the presence of abnormal adaptive immune responses against self-components. In contrast, auto-inflammatory diseases are described as a group of inherited disorders characterized by episodes of seemingly unprovoked, recurrent inflammatory attacks of innate nature, mainly mediated by neutrophils. Furthermore, no significant high-titer autoantibodies or antigen-specific T cells are present [34]. The last group can be divided in six groups depending on the molecular criteria: IL-18 activation disorders (inflammasomopathies), NF-κB activation syndromes, protein misfolding disorders, complement regulatory diseases, disturbances in

**Discussion**

In our study, we showed a significant IL-18 production ex vivo by cultured patients’ PBMCs. This production was increased when cells where stimulated with PHA. Ex vivo increased IL-18 production has been shown during pulmonary Behçet disease. This production was increased ex vivo when cells were treated with LPS [25]. However, this bacterial component activates innate receptors (TLR2 and TLR4) while the plant lectin PHA act as a mitogen for T lymphocytes therefore activating the adaptive immune responses [26,27]. These results suggest that IL-18 can be induced either during innate and/or adaptive immune responses. As the first name of IL-18 was IFN-gamma inducing factor, we measured IFN-γ in culture supernatants after IL-18 addition. We showed that IL-18 enhanced IFN-γ production ex vivo. This has been shown transcriptionally in patients with pulmonary manifestations [25]. In our work, we focused on the ocular manifestation during Behçet disease. Our results showed a similar response profile suggesting a shared pathological role for IL-18 during the two clinical manifestations. This role could be associated to the polarization to Th1 responses and its sustentation during Behçet disease [28]. In addition, an increasing amount of data is implying the Th17 responses in Behçet disease’s immune pathogenicity [29]. IL-18 can also play an important role during Th17 immune responses. The role of IL-18 in Th17 responses appears to be that of activating/amplifying IL-17 production in already polarized Th17 cells, in a TCR-independent manner in synergy with IL-23, similar to its role in TCR-independent activation of Th1 cells together with IL-12 [30,31].

Several studies have shown the clinical relevance of IL-18 in regulating the inflammatory response in vivo. In fact, this cytokine has been detected in inflammatory fluids/tissues of several autoimmune diseases such as the cerebrospinal fluids...
Corticoids’ effect on IL-18 during Behçet uveitis

Cytokine signalling, and macrophage activation syndromes. The first category implied abnormal activation of the inflammasome, which finally leads to the activation of caspase 1, and the subsequent cleavage of the IL-1β as well as the other IL-1 family cytokines [35]. In our pathological model of Behçet disease, more investigations in this sense are required to situate the disease category in autoimmune pathology and/or auto-inflammatory disease. In their study, Türe-Özdemir et al showed caspase-1 activation in responses to pattern recognition receptor engagement on neutrophils and dendritic cells in Behçet’s disease [36]. This activation leads to the cleavage and the liberation of active IL-1β and IL-18 [21]. Our study underlines the association of IL-18 with the inflammatory process during the ocular lesions. Additionally, we showed the implication of IL-18 in the induction and sustain of Th1 responses by the induction of IFN-γ. Altogether, our results suggest that IL-18 is involved both in inflammatory and probably autoimmune processes during Behçet uveitis in Algerian patients.

IL-18 can be produced by several sources. In fact, it is mainly produced by antigen presenting cells (monocytes, macrophages and dendritic cells) as well as epithelial cells or nervous cells [37]. For the first time, our results showed an inhibitory effect of glucocorticoids on IL-18 during disease active stages both in vivo and ex vivo. In a previous study, we showed a significant inhibitory effect of these anti-inflammatory molecules on NO and IL-8 production, two inflammatory markers associated with disease activity during Behçet uveitis [20]. This could be due to the glucocorticoids’ inhibitory effect on NF-κB, the main transcriptional factor implicated in the induction of all these three molecules [38]. In a recent study, our team showed the over-expression of NF-κB in Behçet patients PBMCs. Increased NF-κB expression is concomitant with the over-expression of iNOS, the enzyme that produce NO [39]. In the same way, Hamzaoui et al. reported increased IL-18 expression when they stimulated patients cells cultures (from broncho-alveolar lavage fluid) with LPS [25]. This component induces the activation of NF-κB [40]. All these data suggest the implication of this signalling pathway in the inhibitory effect of corticotherapy during active stage of Behçet uveitis. In addition to their transcriptional effect, glucocorticoids strongly affect the lymphocytes’ viability and hematopoiesis. In fact, glucocorticoids induce T lymphocytes apoptosis and the switch to granulocyte production in bon morrow [41,42]. Those effects are observed during BD where patients shows increased neutrophil-lymphocyte ratio and neutrophil-lyeucocyte ratio [43,44]. The shift to granulocytes production may be implicated in the reduction of IL-18 production. The obtained results, suggest that IL-18 can serve as a good marker for disease activity and therapy efficiency.

Conclusion

Our results showed IL-18 production increase associated with disease activity in Algerian patients with Behçet uveitis. These facts underline the association of IL-18 with the systemic inflammatory process during the ocular lesions. On the other hand, IFN-γ induction by IL-18 suggest that IL-18 production during early inflammatory responses is responsible, at least in part, of the induction of the adaptive Th1 chronic autoimmune responses and therefore the chronicity of Behçet uveitis in Algerian patients. This cytokine could help the management of the disease by monitoring either activity and pathological immune responses or therapy efficiency. Furthermore, glucocorticoids efficiency in reducing IL-18 during active stage underlines their importance in patients’ treatment. Monitoring IL-18 while giving these molecules during early stage of inflammatory uveitis could prevent the establishment of the disease.

Disclosure of interest

The authors declare that they have no conflict of interest concerning this article.

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