RESEARCH ARTICLE

Free circulating interleukin-18 is increased in Schnitzler syndrome: a new autoinflammatory disease?

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ABSTRACT. Schnitzler syndrome is a rare disease characterised by chronic urticaria and arthralgia. The recent evidence that the IL-1 receptor antagonist IL-1Ra could induce rapid and complete remission of Schnitzler symptoms has pointed to IL-1 as a major pathological factor in this disease. To examine the possibility that Schnitzler syndrome may be considered to be an autoinflammatory disease, in this study we measured the serum levels of IL-18, another cytokine of the IL-1 family that is cleaved by caspase-1, in two recently diagnosed Schnitzler patients before and after treatment with IL-1Ra. In parallel, mRNA expression of IL-1 family cytokines and caspase-1 were assessed in isolated blood monocytes. Treatment with IL-1Ra significantly inhibited IL-1β gene expression, indicating that IL-1β activity in Schnitzler syndrome is central to IL-1β gene upregulation in a type of auto-amplification loop. While no IL-1β was detected in serum, free circulating IL-18 was increased in patients with Schnitzler syndrome, despite low IL-18 gene expression in monocytes. This suggests constitutive activation of the IL-1β/IL-18-producing inflammasome, and supports the hypothesis that Schnitzler's syndrome is a new autoinflammatory disease.

Keywords: interleukin-18, caspase-1, IL-1Ra, autoinflammatory diseases, Schnitzler syndrome

Schnitzler syndrome is a rare disorder (94 cases reported worldwide up to 2007), characterised by chronic urticaria, monoclonal gammopathy, fever, arthralgia/arthritis and bone pain with marked elevation of acute phase reactants [1]. In the long term, 15% of patients develop a lymphoproliferative disorder. Manifestations are usually unaffected by steroid or immunosuppressive treatment. Recently, treatment with IL-1 receptor antagonist (IL-1Ra; anakinra) has proven very effective, raising the issue of the role of the inflammatory cytokine IL-1β in the pathogenesis of the disease [2, 3]. Hyperproduction of IL-1β from LPS-stimulated peripheral blood mononuclear cells from one Schnitzler patient has been reported; the addition to stimulated cells of a caspase-1 inhibitor normalised IL-1 levels, while the monoclonal anti-TNF-α antibody adalimumab was ineffective [4]. This finding leads to the hypothesis that Schnitzler syndrome may be a *bona fide* autoinflammatory disease, in which anomalous activation/regulation of the IL-1β producing inflammasome is at the root of the pathological signs. The IL-1β-producing inflammasome is a cytoplasmic protein complex that reacts to endocellular signals by activating the IL-1β cleaving enzyme caspase-1. Activated caspase-1 cleaves the inactive IL-1β precursor protein (pro-IL-1β) and releases the mature, active form of the cytokine [5]. Recent data have shown that the IL-1β-producing inflammasome also produces another cytokine of the IL-1 family, IL-18, by cleaving its precursor protein with a mechanism identical to IL-1β maturation [5]. Thus, excessive production of IL-18 is an expected feature of inflammasome over-activation in autoinflammatory diseases.

In this study, we examined two patients recently diagnosed as suffering from Schnitzler syndrome, to evaluate possible changes in IL-18 production. Since IL-18 is normally present in serum at detectable levels, this being at variance with IL-1β, which is never detectable (except in septic shock), we measured circulating IL-18 levels in patients before and after treatment with anakinra, in parallel with other inflammatory and anti-inflammatory cytokines. In addition, mRNA expression was assessed in blood monocytes for IL-1β, IL-18, their natural inhibitors IL-1Ra and IL-18BP, and caspase-1. Data obtained showed that indeed IL-18 levels are elevated in Schnitzler patients, and that this increase is abolished after treatment with anakinra. In addition, IL-1β expression in monocytes is dramatically reduced following treatment, suggesting interference by IL-1Ra with the ability of IL-1β to amplify its own expression in an autostimulatory loop [6].

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METHOD

Patients

The first patient, a 45-year-old man, had a three-year history of chronic urticaria, bone pain and fever. He had been treated with steroids, cyclosporine, and antihistamines without success. The second patient, a 69-year-old man, had had recurrent episodes of fever and pruritic urticarial lesions in the last six years. He had been treated with steroids and rituximab, but only high doses of steroids had been found to partially control symptoms. In both patients, an extensive clinical work-up had excluded infectious and neoplastic disorders. Laboratory investigations showed leukocytosis, elevated acute phase reactants and the presence of a monoclonal IgM component. Anakinra treatment was started (monotherapy in the first patient; associated with low dose steroids in the second patient), resulting in the disappearance of fever and urticaria within 24 hr. Normalisation of acute phase reactants was observed within one month, and steroids were discontinued. In both patients, blood samples were taken before starting anakinra and after one month of treatment. Twenty four healthy subjects were the control group. Informed consent was obtained from all the subjects and the study was approved by the Ethical Committee of the S.Chiara Hospital of the University of Pisa.

Cytokine assay and calculation of free IL-18

Commercially available ELISA kits were used for measuring serum levels of IL-18 (MBL International, Woburn, MA, USA), IL-18BP and IL-1β (R&D Systems, Minneapolis, MN, USA). For the ELISA of IL-12, IL-10, IL-1Ra, TNF-α and TGF-β, specific antibody pairs and standards were purchased from R&D Systems. For determining the serum concentration of free, active IL-18 (*i.e.* not bound to its inhibitor IL-18BP), the law of mass action was used, the concentrations of both IL-18 and IL-18BP, the stoichiometric 1:1 relationship, and the affinity of binding of 400 pM being known for each sample (measured in the ELISA) [7].

Isolation of monocytes

CD14+ monocytes were isolated from PBMC (obtained from venous blood after centrifugation on a Ficoll gradient) by magnetic separation with a MACS® cell separation procedure using CD14 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Both monocyte purity and vitality were > 98%.

RNA extraction, retrotranscription and real-time PCR

RNA was extracted from blood $CD14^+$ monocytes using Rneasy kits (Qiagen S.p.A., Milano, Italy), the RNA was purified and retrotranscribed with Quantitect® Reverse Transcription Kit (Qiagen). Real-time PCR analysis of gene expression was performed with the SybrGreen method using a Rotor-Gene™ 3000 device (Corbett Research, Sidney, Australia) and specific primer pairs. Gene expression is reported in arbitrary units (AU), compared to the housekeeping gene β-actin (= 1 000 AU).

Statistical analysis

Data from control subjects are presented as mean ± SEM, while those from patients are single results. The statistical significance of differences between patients' data and control values was assessed using the Mann-Whitney non-parametric test.

RESULTS AND DISCUSSION

The study was conducted in two patients affected by Schnitzler syndrome. It had been possible to examine these patients soon after diagnosis and before initiation of treatment with anakinra, and one month after successful treatment.

As in healthy controls, IL-1Ra was not expressed in Schnitzler monocytes and was not induced after therapy (data not shown). The expression of IL-18BP and caspase-1 was significant and comparable to normal controls both before and after treatment *(figure 1)*. For IL-18BP expression, there was a tendency to lower

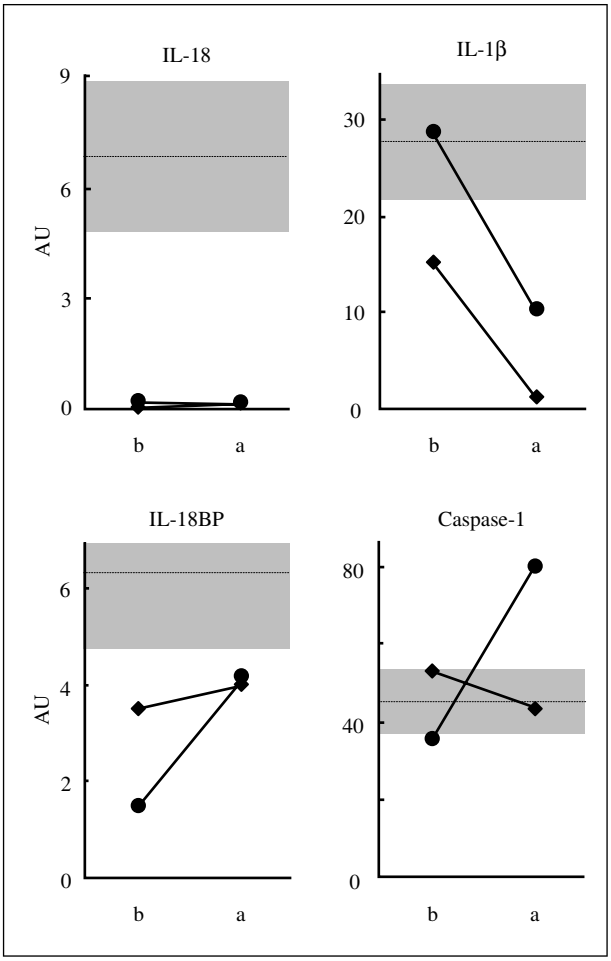

Figure 1
Cytokine gene expression. Gene expression (expressed in arbitrary units) in monocytes from two Schnitzler patients was evaluated before (**B**) and one month after (**A**) treatment with anakinra. Genes examined were IL-18 (upper left), IL-1β (upper right), IL-18BP (lower left), and caspase-1 (lower right). Dotted line indicates the mean value in normal controls (24 matched healthy donors). The grey areas represent SEM above and below the mean of control values.

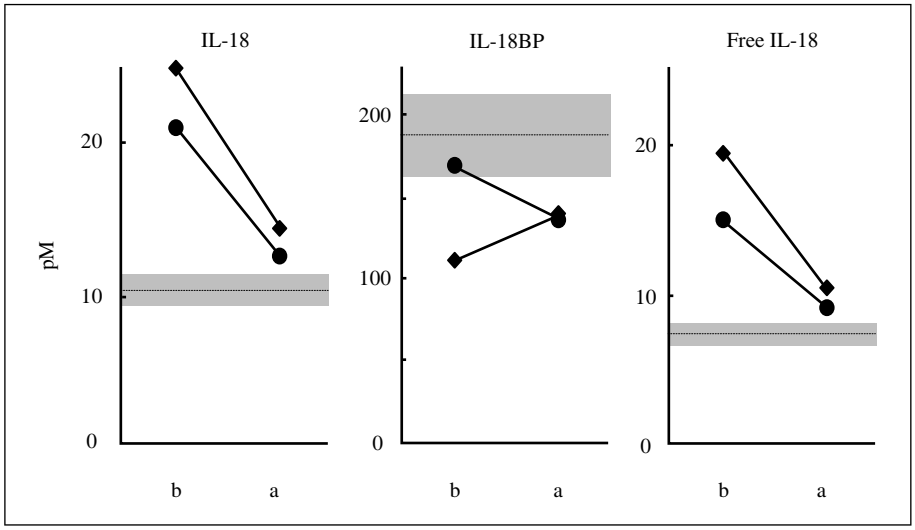

Figure 2
Serum level of total IL-18, IL-18BP, and free IL-18. Values (in pM) for two Schnitzler patients before (**B**) and after (**A**) treatment with anakinra are plotted in comparison with the mean values of normal controls (dotted line). The grey areas represent SEM above and below the mean of control values.

expression in patients as compared to controls, and to an increase after treatment *(figure 1)*. However, due to the wide distribution of IL-18BP expression values in controls, this tendency is not statistically relevant. IL-1β expression was similar to controls before treatment, and was decreased five-fold after anakinra administration *(figure 1)*. This is in agreement with the notion that IL-1 is a potent inducer of IL-1 [6], thus IL-1 activity blockade by anakinra consequently decreases IL-1 expression. This also suggests that the levels of IL-1β expression in Schnitzler patients are largely sustained by IL-1 itself. Expression of other IL-1-related genes (IL-1RI, IL-1RII, IL-1RAcP, TIR8/SIGIRR, IL-1F7, IL-33, T1/ST2) was either undetectable or no different from controls (not shown).

On the other hand, Schnitzler patients had significantly lower expression of IL-18, both before and after treatment (0.18 AU versus 6.8 in controls, $p = 0.02$) *(figure 1)*.

At the serum level, IL-1β was undetectable, as were TNF-α, IL-12, IL-10, and TGF-β. As expected, IL-1Ra was only detectable after treatment (not shown). The levels of free IL-18 were increased in Schnitzler patients as compared to controls (17.2 pM *versus* 7.2 pM in controls, $p = 0.01$), due exclusively to higher levels of total IL-18, whereas the circulating IL-18 inhibitor IL-18BP was slightly lower than in controls *(figure 2)*. Results in a third patient (who could not be included in this study because samples after treatment were not made available) are fully in line with the finding of increased free circulating IL-18 levels (38.1 pM), due to higher IL-18 (49.3 pM *versus* 10.2 pM in controls) with IL-18BP being slightly lower than controls (128.9 pM *versus* 185.5 pM in controls). Free serum IL-18 was decreased after treatment (from 17.2 to 9.9 pM), again exclusively as a consequence of the decrease in total IL-18, with no detectable effect on IL-18BP levels *(figure 2)*.

These data suggest that elevated levels of IL-18 are a prominent feature of Schnitzler syndrome and that they can be reversed by treatment with anakinra. It should be underlined that, at variance with all those autoimmune and inflammatory disorders in which IL-18 has been studied [8-10], no increase in IL-18BP levels was detected in our Schnitzler patients. IL-18BP binds to mature IL-18 with high affinity and prevents its interaction with cell surface receptors [11]. Its production in diseases where elevated IL-18 is detected can be interpreted as a feedback mechanism to limit excessive IL-18 activity. Indeed, in physiological conditions IL-18 induces the synthesis of IFN-γ which, in a feed-back control mechanism, is the major inducer of IL-18BP [12, 13]. Thus, the lack of an increased production of IL-18BP in Schnitzler patients contributes to the elevation of free IL-18 levels. The expression of IL-1-related cytokines (IL-1β, IL-18) and of IL-1-converting enzyme caspase-1 in circulating monocytes was not increased as compared to controls and, in the case of IL-18, it was significantly decreased. The high circulating levels of IL-18 (despite low IL-18 expression and normal caspase-1 expression) suggest that hyperactivity of caspase-1 may be responsible. This evidence indicates that Schnitzler syndrome should be considered as one of the autoinflammatory diseases caused by constitutive activation of the IL-1β/IL-18-producing inflammasome. Experiments are in progress to test this hypothesis.

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