Up-regulation of Interleukin-25 Expression in Vogt-Koyanagi- Harada Disease after Immunosuppressive Treatment

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Research Article

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Abstract

Purpose: IL-25 has been shown to play an *immunosuppressive* role in a series of autoimmune diseases. This study aims to investigate the changes of IL-25 in PBMC in Vogt-Koyanagi-Harada (VKH) patients after the administration of cyclosporine and corticosteroids.

Methods: The protein level of IL-25 in the serum of VKH patients was measured by ELISA. The PBMCs from active VKH patients were cultured in RPMI 1640 medium with 10% fetal bovine serum and stimulated with LPS, CsA or dexamethasone for 3 days. The concentration of IL-25 in supernatants was tested by ELISA respectively.

Results: The protein level of IL-25 is down-regulated in active VKH patients and comparing with inactive patients. The expression of IL-25 was significantly increased in PBMCs from VKH patients after corticosteroid and CsA stimulation.

Conclusion: IL-25 might be a negative regulator of the inflammatory response in VKH syndrome, and the therapeutic effect of corticosteroids and CsA, might upregulate the expression of IL-25 to exert its immunosuppressive effect on VKH syndrome.

Introduction

Vogt-Koyanagi-Harada (VKH) disease is one of the most common forms of uveitis in Asians[1–4].It is an autoimmune disease characterized by a bilateral granulomatous uveitis, which mainly affects the eyes, ears, skin and central nervous system[5]. Although the specific pathogenesis of VKH syndrome is still in the research stage, various types of immune cells have been found to play a vital role in the pathogenesis of VKH disease [6, 7]. Early studies confirmed that macrophages, dendritic cells, Th1 and Th17 cells were related to the pathogenesis of Vogt Koyanagi Harada (VKH) disease [6]. In addition, a variety of inflammatory factors, such as IL-21, IL-27, IL-37, IL-35 and IL-12, have been found to be involved in the pathogenesis of VKH disease [8–12]. Previous studies of our research group showed that IL-25 may participate in the development of VKH disease by inhibiting the expression of proinflammatory cytokines [13].

Interleukin-25 is a newly discovered member of the IL-17 family, localized on chromosome 14q11, and was first reported by Lee et al [14]. It has 3987 base pairs (bp), contains a 483 bp open reading frame, and encodes 161 amino acids [15]. IL-25 has been proved to be produced by a variety of different cells and tissues and has the characteristics of wide distribution [16–25] .The IL-25 receptor (IL-25R) consists of IL-17RA and IL-17RB subunits[26]. Mainly expressed on T cells, NKT cells, ILC2s, endothelial cells, dendritic cells, macrophages and so on [27–32]. Although IL-25 belongs to the IL-17 family, it has only 16% homology with IL-17A, and has a significantly different biological function from other IL-17 family cytokines [17]. On the one hand, IL-25 can induce type 2 immune response and promote the secretion of cytokines such as IL-4, IL-5, IL-9 and IL-13 [33]. On the other hand, it can also bind with IL-17A and its receptor il-17RA to inhibit the formation of pro-inflammatory factors and participate in autoimmune

inflammatory diseases. A previous study showed that IL-25 can reduce the production of pro-inflammatory cytokines IL-12 and IL-23, thereby inhibiting DC driven Th1 inflammatory activity [34]. Study has found that IL-25 primed mesenchymal stem cells can improve the symptoms of inflammatory bowel disease by inhibiting Th17 immune response and inducing T regulatory cell phenotype [35]. In our previous study, we found that compared with the control group, the level of IL-25 in serum of VKH patients was significantly down-regulated. In addition, in vitro experiments have shown that rIL-25 can significantly inhibit the production of IL-1β, IL-6 and TNF-α in PBMC of active VKH patients [13].

The purpose of this study was to confirm the discovery of IL-25 in VKH disease and to further find out the changes of IL-25 expression in PBMC of VKH patients after the treatment with corticosteroid or CsA.

Materials And Methods

Patients

Blood samples from patients and controls were collected at the First Affiliated Hospital of Chongqing Medical University from September 2017 to April 2021. The study comprises 8 active VKH patients(3 males and 5 females, average age = 37 years), 8 inactive VKH patients(4 males and 4 females, average age = 35 years),and 8 healthy controls (5 males and 3 females, average age = 37years). The diagnosis of VKH syndrome is based on the revised VKH diagnostic criteria of the International Nomenclature Committee [36]. Blood samples were collected from patients with active VKH who had not taken medication for at least 1 weeks or or only used a very low dose of oral prednisone (< 20 mg/d). Inactive VKH patients showed no active intraocular inflammation (inactive uveitis stage) for at least 3 months before blood collection. All procedures strictly complied with the principles of the Declaration of Helsinki and were authorized by the Clinical Research Ethics Committee of Chongqing Medical University. Informed consent was acquired from all subjects before blood collection.

Cell isolation and culture

Peripheral blood mononuclear cells (PBMCs) were separated from heparinized blood by Ficoll-Hypaque density gradient centrifugation and cultured in a Roswell Park Memorial Institute 1640 (RPMI 1640) medium which was supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. PBMCs were seeded at a density of $1*10^6$ cells/ mL in 24-well plates. To analyze the effect of corticosteroids and CsA on IL-25 expression in vitro, PBMCs from 8 active VKH patients and 8 healthy volunteers were stimulated with 100 ng/mL LPS in the absence or presence of 500 ng/mL dexamethasone (MCE) or 100 ng/mL CsA (MCE) for 72 h .

ELISA

The concentration of IL-25 in the serum of patients and controls was assayed using a human interlrukin25(IL-25) enzyme-linked immunosorbent assay (ELISA) kit (Peprotech,Rocky Hill, NJ, USA) with

a detection range of 32–2000 pg/mL.

Statistical Analysis

The Shapiro-Wilk normality test, the F-test, the Mann Whitney test, independent-samples t test,and paired-samples t test were applied using SPSS 12.0. Data are expressed as means±SEM. $p<0.05$ was considered to be statistically significant.

Results

The concentration of IL-25 in serum decreased in active VKH patients.

IL-25 was detected in serum samples from patients with VKH disease and controls. The level of IL-25 in patients with active VKH disease was significantly lower than that in controls ($p<0.01$) (Figure 1.A) and in patients with inactive VKH disease($p<0.05$) (Figure 1.B) .There was no significant difference in serum IL-25 level between patients with inactive VKH disease and controls.

The expression of IL-25 increased in VKH patients after treatment with corticosteroids or CsA.

Since corticosteroids and cyclosporine A are commonly used drugs for the effective treatment of VKH diseases, we conducted a preliminary experiment to study the effects of these two drugs on the expression of IL-25. To clarify which of the 2 drugs regulated the production of IL-25, PBMCs from 8 VKH patients were stimulated with LPS in the presence or absence of dexamethasone or CsA for 72h. The results showed that dexamethasone significantly enhances the expression of IL-25 in PBMCs ($p<0.05$) (Figure 2.A) and that CsA has a similar effect ($p<0.05$) (Figure 2.B).

Discussion

In this study, we reconfirmed that IL-25 expression in serum was lower in active VKH patients than inactive VKH patients and healthy controls. And further evidence that treatment with CsA and corticosteroids was associated with an increased IL-25 expression in vitro.

A series of studies showed that IL-25 was associated with Th1/Th17 response [37, 38]. Vogt-Koyanagi-Harada syndrome is perceived to be mediated by Th1 and Th17 cell [6, 37, 39]. In order to confirm whether IL-25 is involved in the pathogenesis of VKH, we first compared the expression of IL-25 in VKH patients and normal controls. The results showed that the expression of IL-25 in serum of patients with active VKH decreased significantly. It indicated that the decrease of IL-25 expression may be related to the development of VKH disease, which is consistent with previous studies by our team[13]. However, this finding is inconsistent with the results of some studies, which show that IL-25 expression in the aqueous

humor of patients with uveitis is increased, and the expression of IL-25 gene is increased [40, 41]. This difference in results may be because IL-25 is involved in the progression of Vogt Koyanagi-Harada disease through more than one mechanism of action. Of course, this hypothesis is still speculative and needs to be confirmed by further research. Unexpectedly, there was no significant difference in IL-25 expression between inactive VKH patients and healthy controls. These results seem to suggest that increased expression of IL-25 correlated with VKH disease activity.

Corticosteroids and CsA are widely used in the treatment of immune-mediated inflammatory diseases and have been shown to be very effective for intraocular inflammation in patients with Vogt-Koyanagi-Harada syndrome[42, 43].In order to better understand whether the two drugs can control the expression of IL-25 and further compare their relative efficacy in regulating IL-25 production, we conducted a series of in vitro experiments. The results of this study show that corticosteroids and CsA can effectively improve the expression of IL-25 in PBMC of VKH patients. This result confirmed that IL-25 may be an inhibitor of VKH syndrome, which is consistent with the results of previous studies. This is also the innovation of this study. It is the first time to propose the relationship between these two drugs and IL-25 in VKH syndrome.

However, it is essential to point out that this study has its limitations. Although we have proved that IL-25 increased after corticosteroids and CsA treatment of VKH syndrome, its specific regulatory mechanism still needs to be further explored and studied. In addition, we studied the changes of IL-25 in PBMC in VKH syndrome, but it is still worth finding out which type of immune cell secretion increases.

In conclusion, this study showed that the decrease of IL-25 level is related to the development of VKH (VKH) disease. It was further found that corticosteroids and cyclosporin may play an immunosuppressive role by regulating the expression of IL-25. At present, there are few studies on the relationship between VKH disease and IL-25.This study can provide new ideas for the study of the pathogenesis of VKH syndrome and provide new directions for the study of the specific mechanism of corticosteroids and cyclosporine in the treatment of VKH syndrome.

Declarations

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Conflict of interest:

The authors Meiqi Gan, Shenglan Yi, Tingting Liu, Yuqin He,Mei Xu all declared that there was no conflict of interest.

Author Contributions:

MG contributed to research, conception and design; MX

contributed to financial support and provision of study materials. MG ,SY, YH and TL contributed to collection and assembly of data. MG, SY and MX contributed to data analysis and interpretation and manuscript writing. MG and MX contributed to final approval of manuscript.

Ethical approval:

All procedures performed in studies involving human participants were following ethical standard and Institutional Review Board, as well as the 1964

Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent:

Informed consent was obtained from all individual participants included in the study.

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Figures

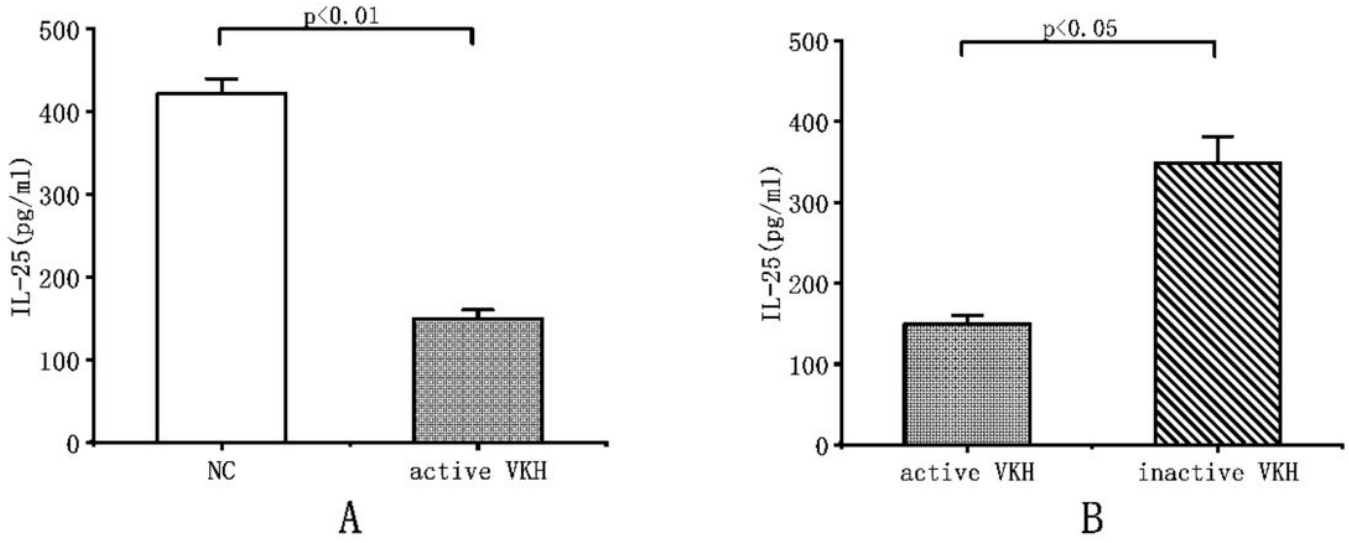

Figure 1

The concentration of IL-25 in serum decreased in active VKH patients. IL-25 levels in serum samples from active VKH patients (n = 8), inactive VKH patients (n= 8), and healthy controls (n =8) were detected by enzyme-linked immunosorbent assay. Kruskal-Wallis test and Mann-Whitney test were used for statistical analyses. Data are expressed as average ± standard deviation ,*p < 0.05, **p < 0.01.

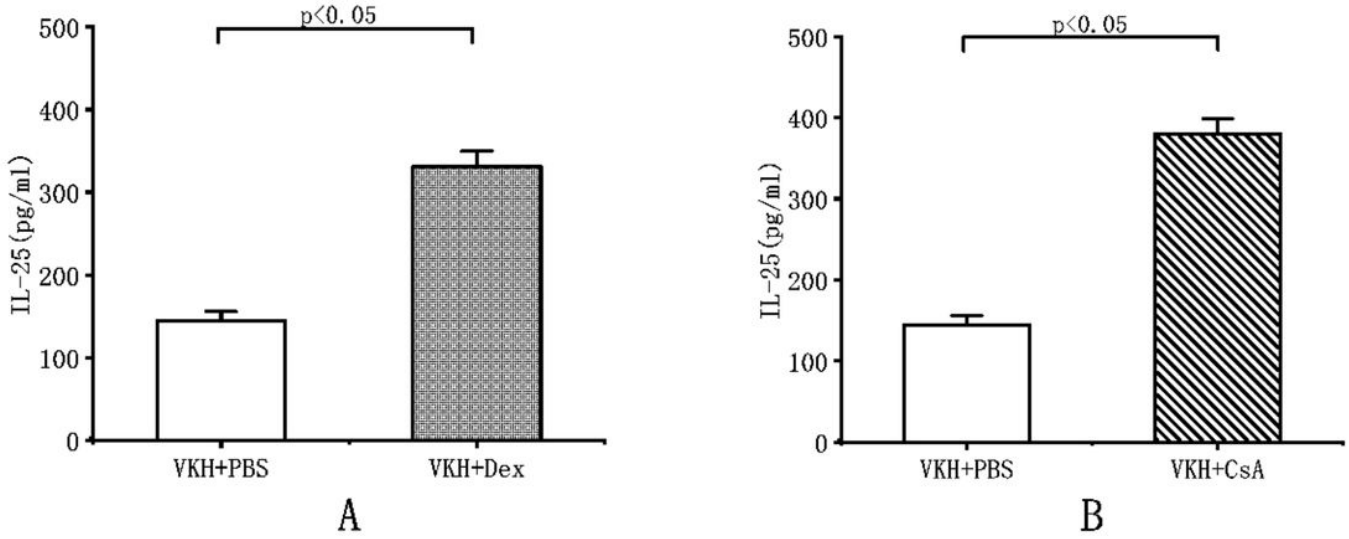

Figure 2

The expression of IL-25 increased in VKH patients after treatment with corticosteroids or CsA. PBMCs were isolated from 8 active VKH patients and 8 healthy volunteers and were stimulated with 100 ng/mL LPS in the absence or presence of 500 ng/mL dexamethasone (MCE) or 100 ng/mL CsA (MCE) for 72 h .The concentration of IL-25 in the supernatants was assayed by ELISA. Kruskal-Wallis test and Mann-Whitney test were used for statistical analyses. Data are expressed as average ± standard deviation ,*p < 0.05.