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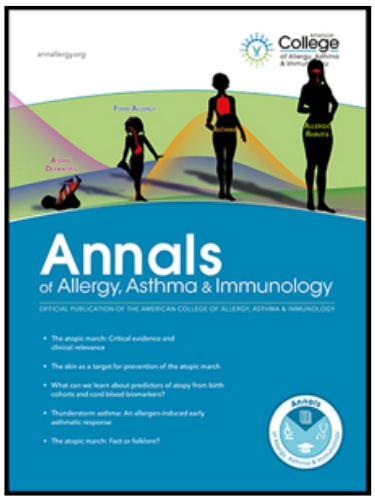

Decreased interleukin-35 serum levels in patients with chronic spontaneous urticaria

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Decreased interleukin-35 serum levels in patients with chronic spontaneous urticaria

Running title: Decreased IL-35 levels in patients with CSU

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Conflict of interest statement

The authors declare no conflict of interest.

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Abbreviations:

CSU: chronic spontaneous urticarial

IL: Interleukin

EBI3: Epstein-Barr virus-induced gene 3

AD: atopic dermatitis

ELISA: enzyme-linked immunosorbent assay

HMC-1: human mast cell line

PMA: phorbol 12- myristate 13-acetate

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Chronic spontaneous urticaria (CSU), a mast cell-driven disease, is defined as the spontaneous appearance of wheals, angioedema or both for > 6 weeks for unknown or known causes[1]. Mast cells, as one of the major effector cells in the immune response system, play

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important roles in the pathogenesis of CSU. Interleukin (IL)-35 is a new anti-inflammatory cytokine which belongs to the IL-12 family and is composed of two subunits: Epstein-Barr virus-induced gene 3 (EBI3) and IL-12p35. IL-35 is widely recognized as a definite immune suppressor with a huge potent of suppression. Abundant evidence had indicated that IL-35 could suppress the activity of Th1 and Th17 cells and plays a crucial role in the pathogenesis of many types of autoimmune diseases, such as inflammatory bowel disease[2], rheumatoid arthritis [3] and systemic lupus erythematosus [4]. However, the effects of IL-35 in the pathogenesis of CSU has not yet been elucidated.

To explore the role of IL-35 in pathogenic mechanism of CSU, this study investigated the serum levels of IL-35 in 40 patients with CSU, 30 with atopic dermatitis (AD), and 40 healthy controls. We also compared the changes of IL-35 serum levels in patients before and after H1 antihistamine treatment. Human researches were approved by the Ethics Committee of Chengdu Second People's Hospital. All patients were oriental, and gave written informed consent. The 40 patients with CSU which enrolled in this study were all the first time to see a doctor and diagnosed with CSU without any treatment before. The severity of CSU was evaluated according daily Urticaria Activity Scores[5] (mean 4.31, range 1-6). The disease severity in patients with AD

was determined by the Scoring of Atopic Dermatitis index[6] (mean 39.26, range 18.8-82.5). All patients and healthy subjects were not treated with any medicines two weeks before this study. Serum samples from all patients or controls were obtained. Among them, 20 patients with CSU were treated with oral H1 antihistamine (cetirizine or fexofenadine) at the licensed dose for 2 weeks. Serum IL-35 levels in different groups were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Catalog Number. CSB-E13126h; Cusabio Biological Manufacture Co. Ltd., Wuhan, China) following the manufacturer's instruction. As showed in Figure1, patients with CSU (73.46 ± 9.146 ng/ml) have significantly lower IL-35 serum levels than AD patients (1264 ± 186.9 ng/ml) and control subjects (1349 ± 170.7 ng/ml). Moreover, we also measured the serum IL-35 levels of CSU patients before and after H1 anti-histamines treatment. The IL-35 levels of 20 CSU patients after H1 anti-histamines treatment (216.80 ± 36.01 ng/ml) were obviously increased when compared with them before treatment (73.46 ± 9.15 ng/ml) ($p = 0.0004$, as shown in Figure1). The serum IL-35 levels were significantly decreased in patients with CSU compared with healthy controls and CSU patients after conventional treatment, which indicated that the reduction of IL-35 levels may play an important role in the pathogenesis of CSU. However, the effects of IL-35 in the pathogenesis of CSU has not

yet been elucidated. To better establish the relevance of IL-35 pathway in CSU, we will investigate the inhibited effects of IL-35 on phorbol 12- myristate 13-acetate (PMA) plus A23187 (calcium ionophore)- stimulated human mast cell line (HMC-1) cells in further study. Mast cells, as one of the major effector cells in the immune response system, are the major primary effector cell type in diseases of immediate hypersensitivity[7], including urticaria and angioedema. Previous studies have indicated that the reduction of the number of mast cells is one of the key indicators of reduced inflammatory symptoms [8]. To explore the role of IL-35 in the HMC-1, Future work is planned to investigate the role of IL-35 in mast cell proliferation and elucidate the intracellular signaling mechanisms that may be involved.

To summarize our results, this study provides first observations on the association of IL-35 and CSU, and showed the decreased IL-35 serum levels in CSU patients. Moreover, we also found that the serum IL-35 levels were significantly increased in CSU patients after conventional treatment. Therefore, we suggest that IL-35 may serve as anti-inflammatory cytokines, and play a role in the pathogenesis of CSU.

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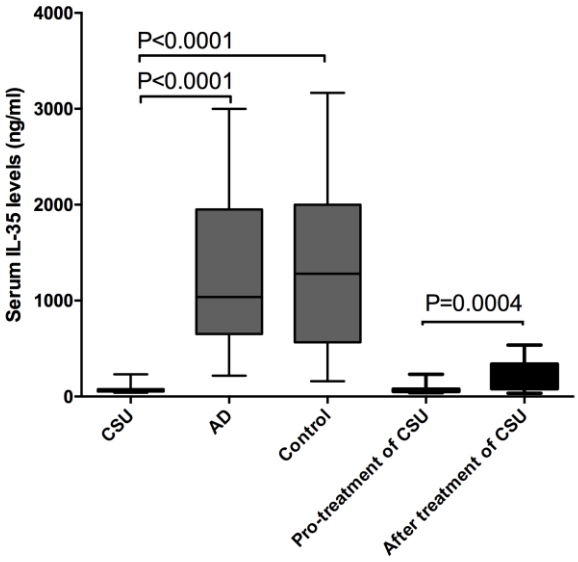

Figure1: Serum levels of IL-35 in patients with CSU and AD and healthy controls were determined by ELISA. Prior to treatment, serum IL-35 levels in patients with CSU were significantly lower than those in AD patients and control subjects. P values are based on the Mann-Whitney U Test. Wilcoxon Signed Ranks Test for paired data showed that the serum IL-35 levels of 20 CSU patients after H1 anti-histamines treatment were significantly higher than those before treatment. All data are expressed as median ± interquartile range. A P value less than 0.05 was set as statistically significant.

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