

Altered serum levels of IL-36 cytokines (IL-36 α , IL-36 β , IL-36 γ , and IL-36Ra) and their potential roles in Guillain-Barré syndrome

Zhikang Zhao, MD^a, Rui Zhang, MD, PhD^a, Xinxin Gao, MD^b, Hui Li, MD^b, Hongbo Liu, MD, PhD^{b,*}

Abstract

Guillain-Barré syndrome (GBS) is an acute autoimmune neurological disorder mainly involving the peripheral nerves. Currently, various cytokines have been shown to be involved in the pathogenesis of GBS. Because of their similar biological structures, interleukin (IL)-36 α , IL-36 β , IL-36 γ , and IL-36 receptor antagonist (Ra) were all renamed and collectively called IL-36 cytokines. The roles of IL-36 cytokines in GBS currently remain unclear.

Forty-two patients with GBS and 32 healthy volunteers were included in our study. Serum IL-36 α , β , γ , and interleukin-36 receptor antagonist (IL-36Ra) levels of patients with GBS in the acute and remission phases and healthy volunteers were measured by enzyme-linked immunosorbent assay (ELISA). In addition, we examined the serum levels of other inflammatory factors that have been shown to be involved in GBS pathogenesis, represented by IL-17 and tumor necrosis factor- α (TNF- α). Furthermore, the correlations between the serum levels of IL-36 cytokines and different clinical data or the serum levels of other inflammatory factors in GBS patients were analyzed.

Significantly higher serum IL-36 α and IL-36 γ levels were measured in the acute phase than in the remission phase and in healthy control (HC) subjects ($P < .05$), while lower serum IL-36Ra levels were measured in the acute phase than in the remission phase and in HC subjects ($P < .05$). Serum IL-36 α and IL-36 γ levels were positively correlated with GBS disability scale scores (GDSs), while serum IL-36Ra levels were negatively correlated with GDSs. Correlation analyses among inflammatory factors showed that serum IL-36 α and IL-36 γ levels in GBS patients were positively correlated with serum IL-17 and TNF- α levels, while serum IL-36Ra levels were negatively correlated with the levels of these 2 inflammatory factors. Similar results were observed in cerebrospinal fluid (CSF), IL-36 α and IL-36 γ levels in CSF were positively correlated with GDSs, while IL-36Ra levels in CSF were negatively correlated with GDSs. Additionally, the serum and CSF levels of IL-36 α and IL-36 γ in the axonal subtype of GBS patients were higher than those in the demyelination subtype.

Based on our findings, IL-36 cytokines may be involved in the pathogenesis of GBS and some of these cytokines may help predict the disease severity and other clinical characteristics of GBS.

Abbreviations: CJ = *Campylobacter jejuni*, CSF = cerebrospinal fluid, DC = dendritic cell, GBS = Guillain-Barré syndrome, IL-36Ra = interleukin-36 receptor antagonist, Th1 = T helper 1, TNF- α = tumor necrosis factor- α .

Keywords: cytokines, Guillain-Barré syndrome, interleukin-36, serum

Editor: Jianli Tao.

This work was supported by the Natural Science Foundation of Henan Province (No. 162300410308).

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

^a Department of Emergency, ^b Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China.

* Correspondence: Hongbo Liu, Department of Neurology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, China (e-mail: liuhongbo6279@126.com).

Copyright © 2020 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the Creative Commons Attribution License 4.0 (CCBY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Zhao Z, Zhang R, Gao X, Li H, Liu H. Altered serum levels of IL-36 cytokines (IL-36 α , IL-36 β , IL-36 γ , and IL-36Ra) and their potential roles in Guillain-Barré syndrome. *Medicine* 2020;99:52(e23832).

Received: 31 May 2020 / Received in final form: 22 October 2020 / Accepted: 19 November 2020

<http://dx.doi.org/10.1097/MD.00000000000023832>

1. Introduction

Guillain-Barré syndrome (GBS) is an acute autoimmune neurological disorder that mainly involves the peripheral nerves. It is clinically characterized by limb weakness, weakening, or disappearance of tendon reflexes and progression over several weeks followed by gradual recovery.^[1,2] Originally, nerve demyelination was considered the main pathological mechanism of GBS. However, many patients with pathology limited to axonal degeneration were identified in subsequent studies, and these changes were associated with some special antibodies against gangliosides, such as GM1, GD1a, and GQ1b, particularly after *Campylobacter jejuni* (CJ) infection.^[3] Currently, GBS is broadly divided into 2 main subtypes: the demyelination subtype and the axonal subtype. The demyelination subtype mainly refers to acute inflammatory demyelinating polyneuropathy (AIDP), which is more common in Western Europe and the United States. The axonal subtypes, consist of 2 common variants: acute motor axonal neuropathy (AMAN) and acute motor and sensory axonal neuropathy (AMSAN), which are associated with prominent axonal injuries and are relatively more prevalent in China, Japan, and Mexico.^[4,5] Currently, the

exact etiology of GBS still remains unclear. It may be related to a variety of factors such as microbial infections, host susceptibilities, and a disruption in the immune balance.^[6–9]

Cytokines often bridge components of the immune system and play crucial roles in initiating, propagating, or regulating autoimmune injuries. According to recent studies, various cytokines, such as tumor necrosis factor- α (TNF- α), IL-17, IL-22, IL-27, and CXCL10, are involved in the pathogenesis of GBS.^[10,11] The IL-36 family is a very interesting group of newly named cytokines derived from the IL-1 cytokine superfamily, including 3 agonists (IL-36 α , β , and γ) and 1 natural receptor antagonist (interleukin-36 receptor antagonist (IL-36Ra)).^[12] Because of their similar biological structures and shared receptor (IL-36R), these cytokines were eventually collectively referred to as IL-36 cytokines.^[13,14] IL-36 agonists play proinflammatory roles by binding to IL-36R and activating the downstream transcription factor nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinase (MAPK) signaling pathway.^[15] Conversely, IL-36Ra suppresses inflammatory responses by competitively binding to IL-36R and inhibiting downstream signaling.^[16] IL-36 cytokines are expressed in many tissues and cells, such as the brain, skin, lungs, gut, dendritic cells (DCs), macrophages, and lymphocytes.^[13,17,18] Recent studies have indicated that IL-36 cytokines may be involved in a variety of inflammatory diseases, such as rheumatoid arthritis, psoriasis, and immune thrombocytopenia.^[14,19–21] However, the roles of IL-36 cytokines in GBS currently remain unclear.

In our study, we determined the serum levels of IL-36 cytokines in patients with GBS during different phases and healthy controls (HCs). Meanwhile, we explored the potential relationships of serum IL-36 cytokines levels with clinical parameters and other inflammatory factors in patients with GBS.

2. Materials and methods

2.1. Study subjects

All subjects were recruited from the Department of Neurology, at the First Affiliated Hospital of Zhengzhou University, China. We tried to include as many suitable patients as possible, and 65 patients with GBS were recruited from July 2016 to August 2019. These patients were diagnosed according to the 1990 Asbury and Cornblath criteria. The exclusion criteria included recent infectious diseases, other autoimmune diseases, malignant neoplasms, and severe liver and kidney dysfunction. If these patients met one or more of the exclusion criteria or failed to complete follow-up, their data were excluded. Finally, 42 patients were included in this study and their serum samples were collected in different phases. Additionally, 32 age- and sex-matched healthy volunteers were included as the HCs. The protocols were performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. Prior to participation, written informed consent was obtained from all participants.

2.2. Clinical assessment

Demographic features, clinical symptoms, electrophysiology, serologic testing, GBS disability scale scores (GDSs), cerebrospinal fluid (CSF) parameters, and therapeutic data were collected. Two neurologists with relevant qualifications independently evaluated GDSs to reflect the disease severity of patients with

GBS. Serum samples were collected from patients with GBS in the acute phase at 9 ± 3 days from disease onset prior to treatment with intravenous immunoglobulin (IVIG) or plasma exchange (PE). Serum samples were collected in the remission phase at 1 to 3 months after preliminary recovery. Because most healthy volunteers and patients in the remission phase were reluctant to undergo lumbar puncture, we only collected CSF samples in the acute phase before immunotherapy. The levels of protein in CSF were measured using immunoturbidimetry and the white blood cell (WBC) counts in CSF were determined using the manual microscopic counting method. All acquired samples were collected and stored at -80°C until use.

2.3. Measurement of cytokine levels

The levels of IL-36 α , IL-36 β , IL-36 γ , IL-36Ra, IL-17, and TNF- α were measured using commercially available human IL-36 α , IL-36 β , IL-36 γ , IL-36Ra, IL-17, and TNF- α enzyme-linked immunosorbent assay (ELISA) kits provided by Shanghai Enzyme-linked Biotechnology Co., Ltd. (Shanghai, China), according to the manufacturer's instructions. The absorbance values of the corresponding substrate were recorded at 450nm. The levels of these cytokines were calculated from standard curves, and the lowest detectable levels were 10.0 pg/ml, 10.0 pg/ml, 10.0 pg/ml, 8.5 pg/ml, 12.5 pg/ml, and 12.5 pg/ml, respectively.

2.4. Statistical analyses

Data are presented as means \pm standard deviations or medians (P25, P75). Differences in variables were analyzed using paired or unpaired *t* tests. Pearson's or Spearman's correlation coefficients were calculated to analyze correlations. A two-tailed *P*-value was considered statistically significant at $<.05$. SPSS 24.0 software (SPSS, IBM, West Grove) was used for statistical analyses.

3. Results

3.1. Demographic and clinical characteristics of study subjects

The demographic and clinical features of the 42 patients with GBS included in this study are displayed in Table 1. No significant differences were observed in the age at sample collection or gender ratio between the patients with GBS and HCs ($P > .05$). Clinical subtypes are divided into demyelination and axonal subtype.

Table 1

Demographic and clinical characteristics of Guillain-Barré syndrome patients and healthy controls.

Clinical characteristics	GBS (n=42)	HC (n=32)
Age (yr)	38.86 \pm 12.52	38.97 \pm 11.65
Gender (F:M)	18:24	14:18
Subtype (D:A)	27:15	Not applicable (NA)
GDSs at sampling prior to treatment	3.00 (2.38, 4.00)	NA
WBCs in CSF ($10^9/l$)	2.00 (1.00, 5.25)	NA
Protein in CSF (mg/l)	841 (649, 1285)	NA

Data are expressed as the mean \pm standard deviation or median (P25, P75). CSF = cerebrospinal fluid, D:A = demyelination subtype:axonal subtype, F:M = female:male, GBS = Guillain-Barré syndrome, GDS = GBS disability scale score, HC = healthy control, WBC = white blood cell.

3.2. Serum levels of IL-36 cytokines and other inflammatory factors in GBS and HCs

Serum IL-36 α and IL-36 γ levels were significantly increased in patients with GBS during the acute phase compared to the HC group. Additionally, serum IL-36 α and IL-36 γ levels in the recovery phase were decreased compared to those in the acute phase, although they were still higher than those in HCs ($P < .01$, Fig. 1a and c). Meanwhile, serum IL-36 β levels in the acute phase of GBS patients were slightly higher than those in HCs, while there was no significant difference between serum IL-36 β levels of patients with GBS in the recovery phase and these in the acute phase or in HCs ($P = .019$, $P = .297$, and $P = .091$, respectively, Fig. 1b). In contrast, serum IL-36Ra levels were decreased in patients with GBS during the acute phase compared to the HC group. Additionally, serum IL-36Ra levels were increased in the recovery phase compared to the acute phase, but were still lower than those in HCs ($P < .01$, Fig. 1d). The serum levels of other inflammatory factors, represented by IL-17 and TNF- α , were also increased in patients with GBS during the acute phase and decreased in the recovery phase, but were still higher than those in HCs ($P < .01$, Fig. 1e and f).

3.3. Correlations between serum IL-36 cytokines levels and clinical parameters in GBS

To evaluate the potential associations between IL-36 cytokines and GBS, we further explored the correlations between the serum levels of these cytokines and clinical parameters in patients with GBS. Our results showed that serum IL-36 α and IL-36 γ levels in GBS were positively correlated with GDSs ($r = 0.671$, $P < .01$, Fig. 2a; $r = 0.664$, $P < .01$, Fig. 2g, respectively), while the serum levels of IL-36Ra were negatively correlated with GDSs ($r = -0.640$, $P < .01$, Fig. 2j). Additionally, significant correlations between serum IL-36 β levels and GDSs were not observed ($r = 0.281$, $P = .071$, Fig. 2d). Simultaneously, statistically significant correlations were not observed between the serum levels of different IL-36 cytokines and protein levels or white blood cell (WBC) count in the CSF of patients with GBS ($r = 0.082$, $P = .606$, Fig. 2b; $r = 0.296$, $P = .057$, Fig. 2c; $r = 0.212$, $P = .178$, Fig. 2e; $r = 0.155$, $P = .326$, Fig. 2f; $r = 0.096$, $P = .545$, Fig. 2h; $r = 0.099$, $P = .532$, Fig. 2i; $r = -0.031$, $P = .846$, Fig. 2k; $r = -0.237$, $P = .131$, Fig. 2l, respectively).

3.4. Correlations among the serum levels of different IL-36 cytokines in GBS

Pearson's correlation coefficients revealed negative correlations between serum IL-36 α and IL-36 γ levels with serum IL-36Ra levels in GBS patients ($r = -0.478$, $P < .01$, Fig. 3c; $r = -0.562$, $P < .01$, Fig. 3f, respectively). Meanwhile, serum IL-36 α levels were positively correlated with serum IL-36 γ levels ($r = 0.691$, $P < .01$, Fig. 3b). Additionally, statistically significant correlations were not observed between serum IL-36 β levels and serum IL-36 α , IL-36 γ , or IL-36Ra levels in GBS patients ($r = 0.168$, $P = .288$, Fig. 3a; $r = 0.295$, $P = .058$, Fig. 3d; $r = -0.194$, $P = .218$, Fig. 3e, respectively).

3.5. Correlations between the serum levels of IL-36 cytokines and other inflammatory factors in GBS

To determine the possible roles of IL-36 cytokines in GBS, we further explored the correlations between the serum levels of IL-36

cytokines and other inflammatory factors, represented by IL-17 and TNF- α , which have been reported to be involved in the pathogenesis of GBS. Our results showed that the serum levels of IL-36 α and IL-36 γ in GBS were positively correlated with the serum levels of IL-17 or TNF- α ($r = 0.425$, $P < .01$, Fig. 4a; $r = 0.474$, $P < .01$, Fig. 4c; $r = 0.493$, $P < .01$, Fig. 4e; $r = 0.564$, $P < .01$, Fig. 4g, respectively). However, statistically significant correlations were not observed between the serum levels of IL-36 β and the serum levels of IL-17 or TNF- α in GBS patients ($r = 0.064$, $P = .688$, Fig. 4b; $r = 0.093$, $P = .558$, Fig. 4f, respectively). In contrast, the serum levels of IL-36Ra were negatively correlated with the serum levels of IL-17 and TNF- α ($r = -0.435$, $P < .01$, Fig. 4d; $r = -0.339$, $P = .028$, Fig. 4h, respectively).

3.6. Correlations between CSF IL-36 cytokines levels and other parameters in GBS

We further explored the correlations between CSF IL-36 cytokines levels and different parameters in GBS. The results showed that CSF IL-36 α and IL-36 γ levels in GBS were positively correlated with GDSs ($r = 0.627$, $P < .01$, Fig. 5a; $r = 0.639$, $P < .01$, Fig. 5g, respectively), while CSF IL-36Ra levels were negatively correlated with GDSs ($r = -0.650$, $P < .01$, Fig. 5j). Additionally, significant correlations between serum IL-36 β levels and GDSs were not observed ($r = 0.288$, $P = .264$, Fig. 5d). Simultaneously, statistically significant correlations were not observed between the CSF levels of different IL-36 cytokines and protein levels or white blood cell (WBC) count in the CSF of patients with GBS. Furthermore, the CSF levels of IL-36 α and IL-36 γ in GBS were positively correlated with the CSF levels of IL-17 and TNF- α ($r = 0.395$, $P = .01$, Fig. 5b; $r = 0.470$, $P < .01$, Fig. 5c; $r = 0.520$, $P < .01$, Fig. 5h; $r = 0.562$, $P < .01$, Fig. 5i, respectively). However, statistically significant correlations were not observed between the CSF levels of IL-36 β and the serum levels of IL-17 or TNF- α in GBS patients ($r = 0.135$, $P = .393$, Fig. 5e; $r = 0.284$, $P = .068$, Fig. 5f, respectively). In contrast, the CSF levels of IL-36Ra were negatively correlated with the CSF levels of IL-17 and TNF- α ($r = -0.438$, $P < .01$, Fig. 5k; $r = -0.356$, $P = .021$, Fig. 5l, respectively).

3.7. Serum and CSF levels of IL-36 cytokines in different clinical subtypes of GBS

To verify whether IL-36 cytokines are associated with the clinical subtype of GBS, we compared the serum levels of IL-36 cytokines in different clinical subtypes. The results showed that serum IL-36 α and IL-36 γ levels in patients with the axonal subtype of GBS were higher than those in patients with the demyelination subtype ($P < .05$, Fig. 6a and b). However, statistically significant differences were not observed in serum IL-36 β and IL-36Ra levels between patients with different clinical subtypes of GBS ($P > .05$, Fig. 6c and d). The CSF levels of IL-36 α and IL-36 γ in patients with the axonal subtype of GBS were higher than those in patients with the demyelination subtype ($P < .05$, Fig. 6e and g), while significant differences in CSF IL-36 β and IL-36Ra levels were not observed between patients with different clinical subtypes of GBS ($P > .05$, Fig. 6f and h).

4. Discussion

Cytokines and their interactions have been demonstrated to be involved in the pathogenesis of GBS.^[10,22] The IL-36 family is an interesting group of newly named cytokines, including IL-36 α , β , γ ,

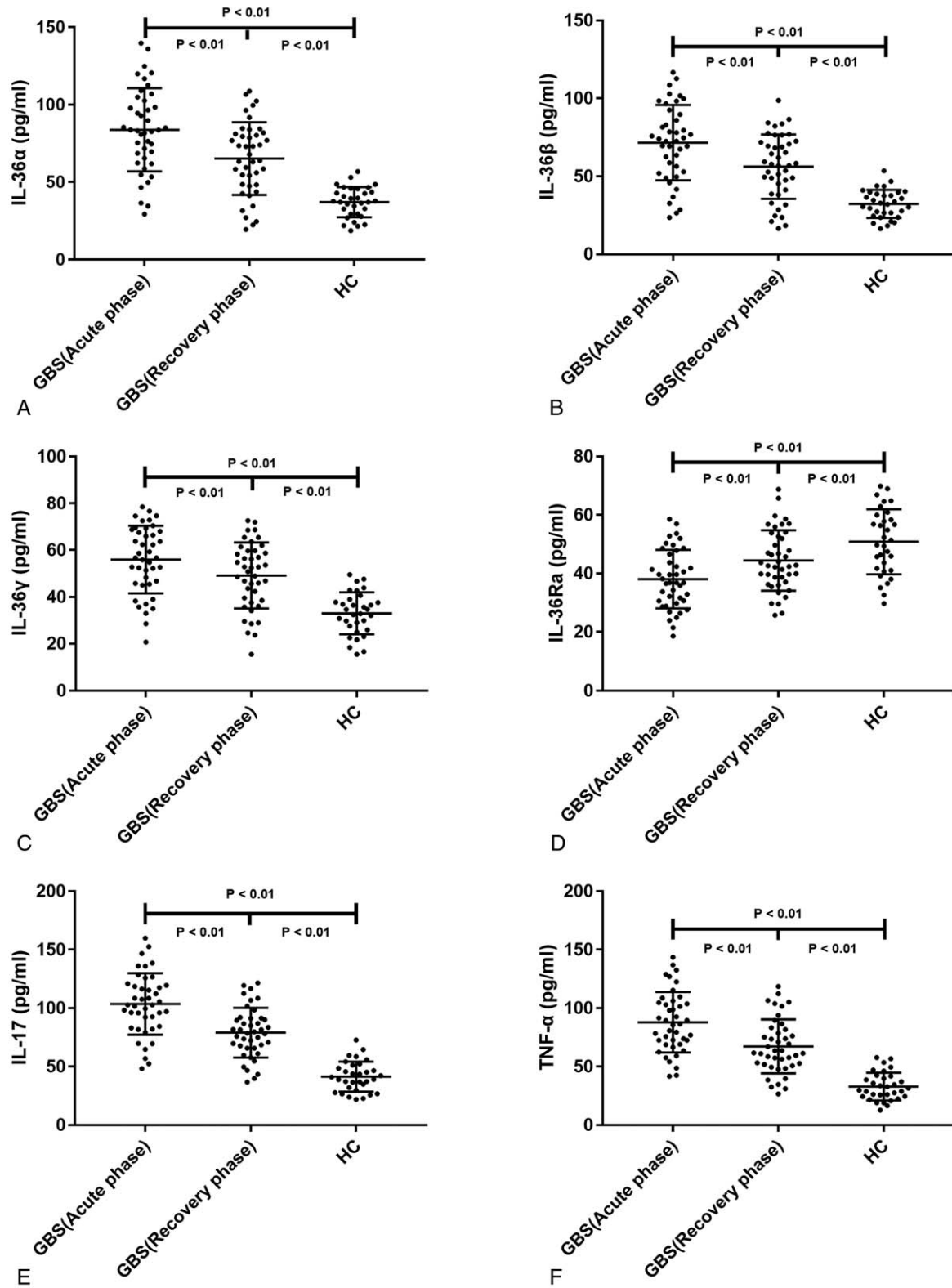


Figure 1. Serum IL-36 cytokines levels in the acute and recovery phase of GBS groups and in HC groups. (a) Serum IL-36α levels in the acute and recovery phase of GBS groups and in HC groups. (b) Serum IL-36β levels in the acute and recovery phase of GBS groups and in HC groups. (c) Serum IL-36γ levels in the acute and recovery phase of GBS groups and in HC groups. (d) Serum IL-36Ra levels in the acute and recovery phase of GBS groups and in HC groups. (e) Serum IL-17 levels in the acute and recovery phase of GBS groups and in HC groups. (f) Serum TNF-α levels in the acute and recovery phase of GBS groups and in HC groups.

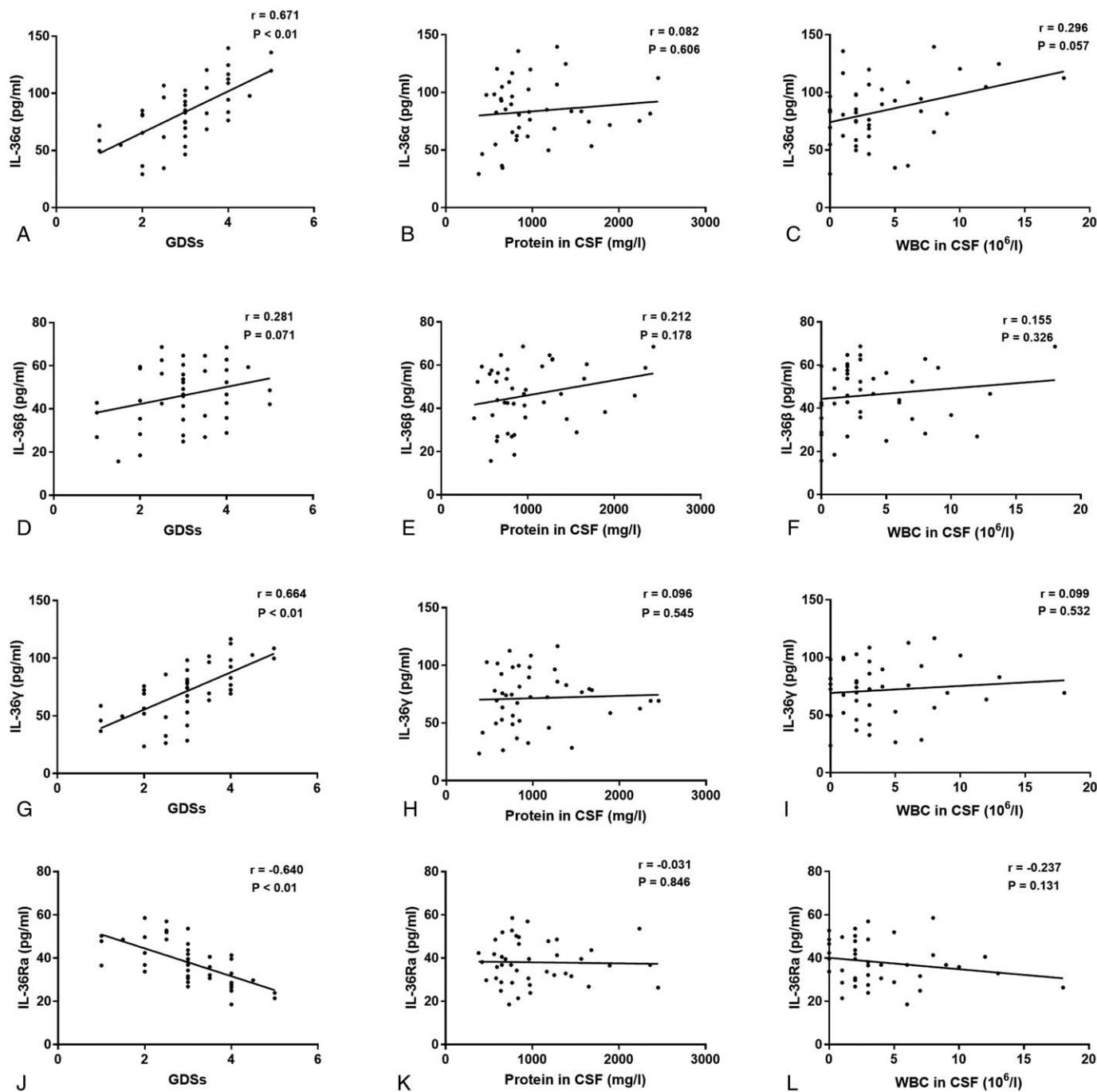


Figure 2. Correlations between the serum levels of IL-36 cytokines and clinical parameters in GBS. (a) The correlation between IL-36α and GDSs in GBS. (b) The correlation between IL-36α and protein in CSF in GBS. (c) The correlation between IL-36α and WBCs in CSF in GBS. (d) The correlation between IL-36β and GDSs in GBS. (e) The correlation between IL-36β and protein in CSF in GBS. (f) The correlation between IL-36β and WBCs in CSF in GBS. (g) The correlation between IL-36γ and GDSs in GBS. (h) The correlation between IL-36γ and protein in CSF in GBS. (i) The correlation between IL-36γ and WBCs in CSF in GBS. (j) The correlation between IL-36Ra and GDSs in GBS. (k) The correlation between IL-36Ra and protein in CSF in GBS. (l) The correlation between IL-36Ra and WBCs in CSF in GBS.

and IL-36Ra. IL-36 cytokines have recently been reported to be involved in the pathophysiological processes of some autoimmune diseases.^[14,23] However, the roles of these cytokines in GBS currently remain unclear. In this study, we performed the first analysis of the possible roles of IL-36 cytokines and their interactions in GBS.

GBS is an autoimmune neurological disorder characterized by limb weakness, weakening, or disappearance of tendon reflexes

and progression over several weeks followed by gradual recovery.^[2] Our results showed that serum IL-36α and IL-36γ levels were significantly elevated in patients with GBS during the acute phase compared to the HC group, and these cytokines tended to decrease in the recovery phase. Conversely, serum IL-36Ra levels were decreased in patients with GBS during the acute phase compared to the HC group, but were increased in the

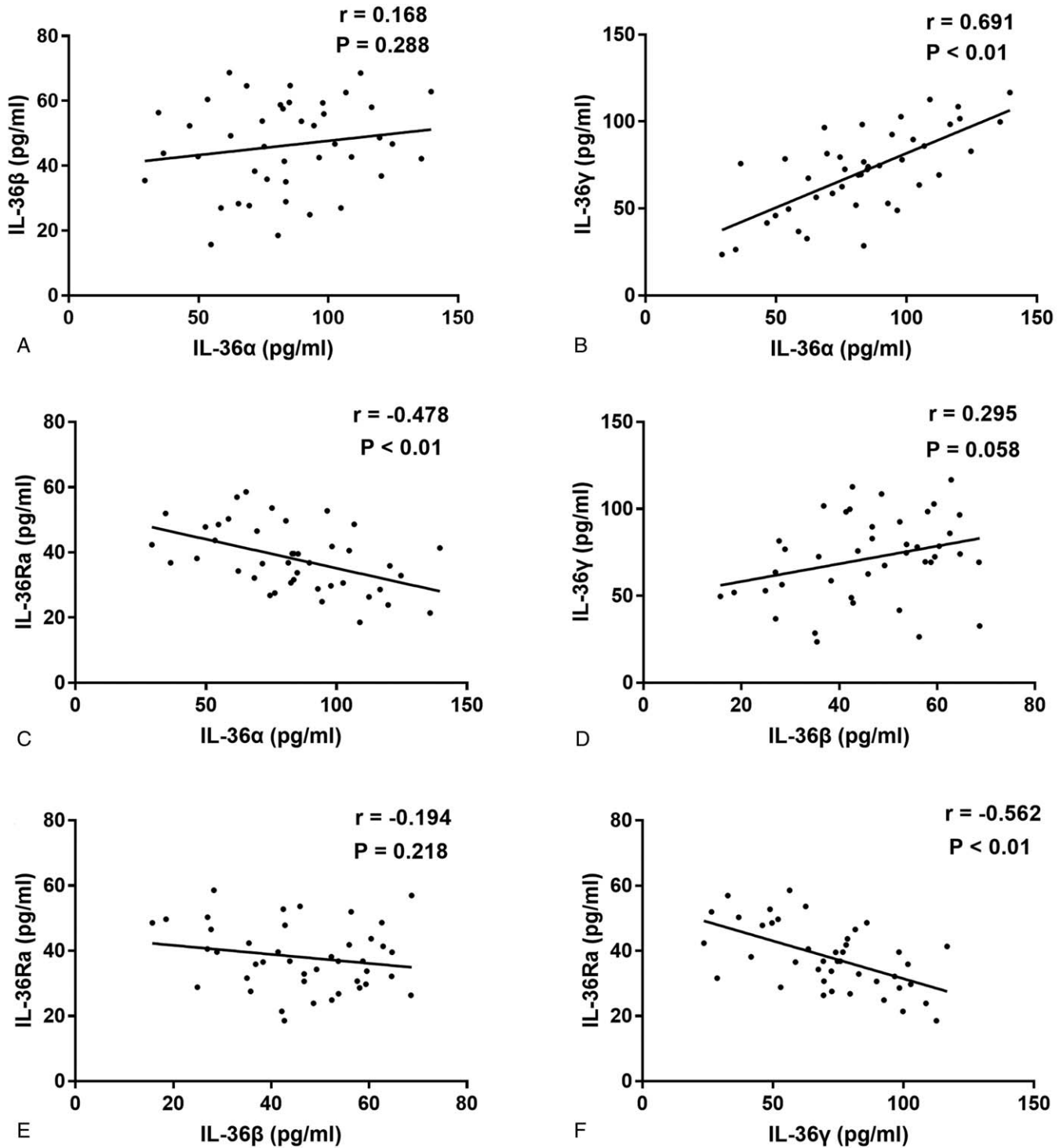


Figure 3. Correlations among the serum levels of different IL-36 cytokines in GBS. (a) The correlation between IL-36 α and IL-36 β in GBS. (b) The correlation between IL-36 α and IL-36 γ in GBS. (c) The correlation between IL-36 α and IL-36Ra in GBS. (d) The correlation between IL-36 β and IL-36 γ in GBS. (e) The correlation between IL-36 β and IL-36Ra in GBS. (f) The correlation between IL-36 γ and IL-36Ra in GBS.

recovery phase, although they were still lower than those in HCs. Based on these results, the altered serum levels of IL-36 cytokines may be potentially associated with the pathological processes of GBS.

IL-36 cytokines are involved in the differentiation of T helper cells 1 (Th1) and Th17 cells, and may affect the expression and

secretion of related cytokines such as TNF- α , IL-17, and IL-22. Meanwhile, these secreted cytokines subsequently stimulate the expression of IL-36 family cytokines, which may form a positive feedback loop.^[24,25] Additionally, IL-36 cytokines also promote the maturation of dendritic cells (DCs) to further promote the differentiation of Th17 cells and the secretion of related

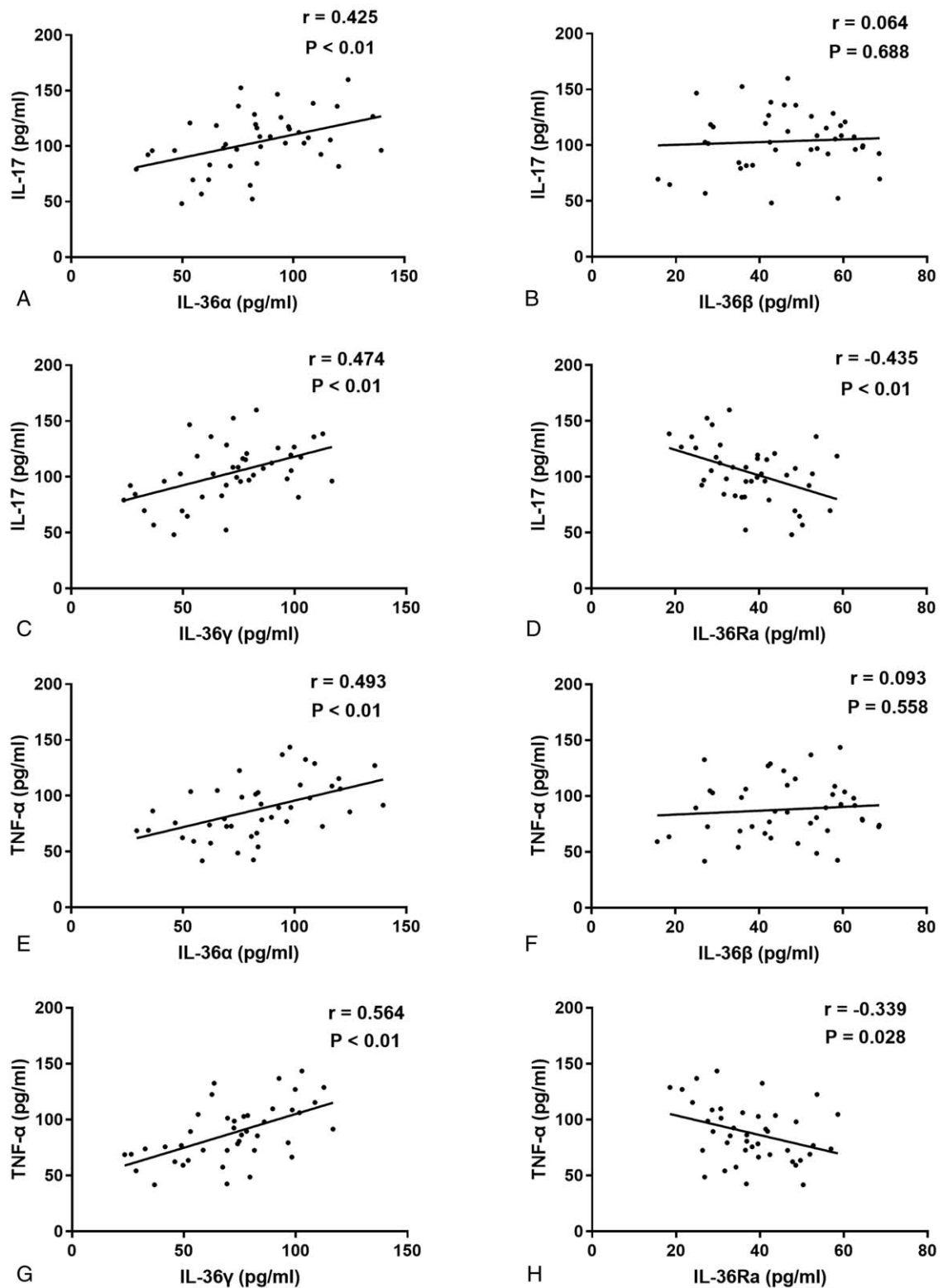


Figure 4. Correlations between serum levels of IL-36 cytokines and serum levels of other inflammatory factors in GBS. (a) The correlation between serum IL-36 α levels and serum IL-17 levels in GBS. (b) The correlation between serum IL-36 β levels and serum IL-17 levels in GBS. (c) The correlation between serum IL-36 γ levels and serum IL-17 levels in GBS. (d) The correlation between serum IL-36Ra levels and serum IL-17 levels in GBS. (e) The correlation between serum IL-36 α and serum TNF- α levels in GBS. (f) The correlation between serum IL-36 β levels and serum TNF- α levels in GBS. (g) The correlation between serum IL-36 γ levels and serum TNF- α levels in GBS. (h) The correlation between serum IL-36Ra levels and serum TNF- α levels in GBS.

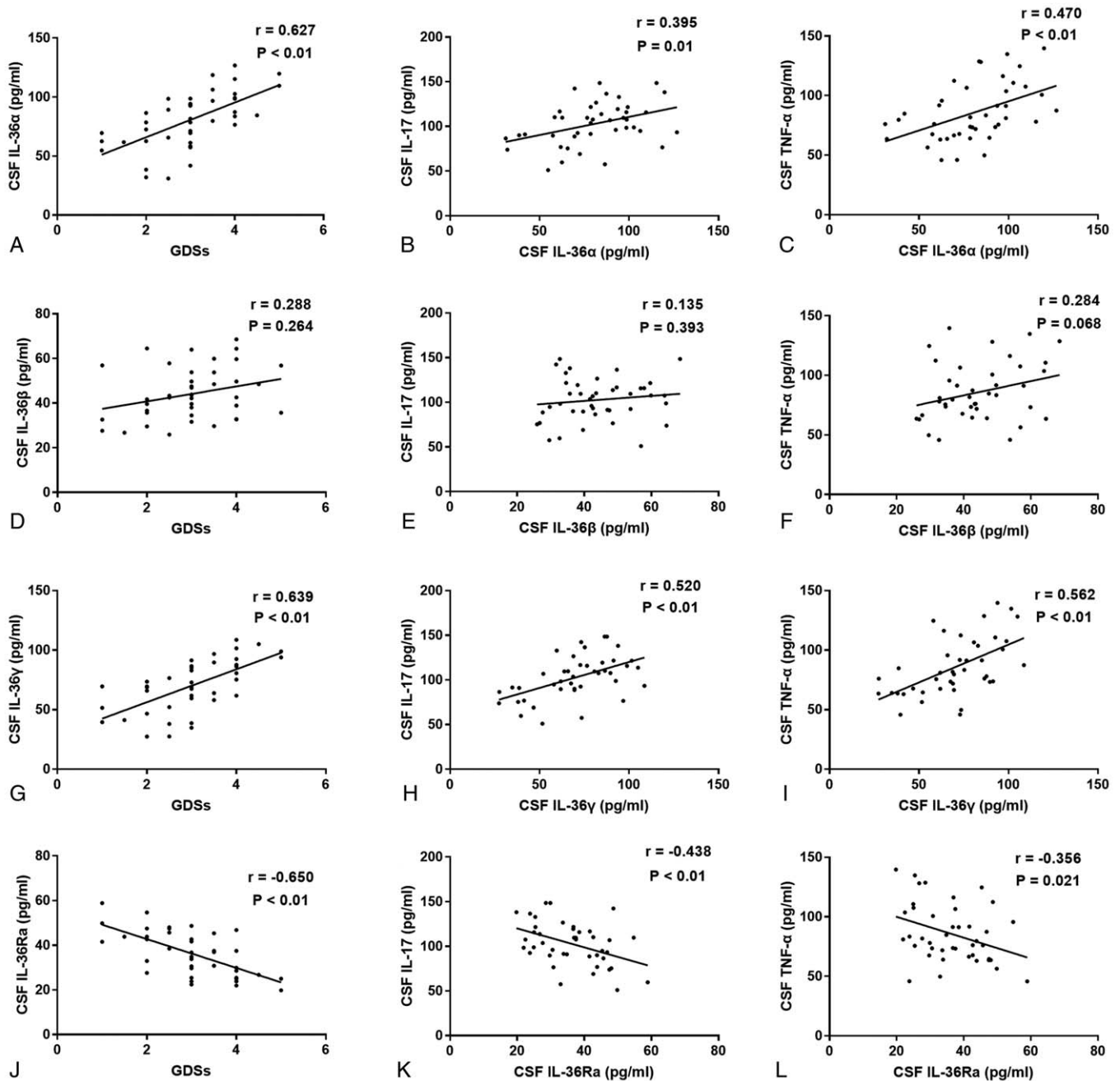


Figure 5. Correlations between CSF IL-36 cytokines levels and different parameters in GBS. (a) The correlation between CSF IL-36 α levels and GDSs in GBS. (b) The correlation between CSF IL-36 α levels and CSF IL-17 levels in GBS. (c) The correlation between CSF IL-36 α levels and CSF TNF- α levels in GBS. (d) The correlation between CSF IL-36 β levels and GDSs in GBS. (e) The correlation between CSF IL-36 β levels and CSF IL-17 levels in GBS. (f) The correlation between CSF IL-36 β levels and CSF TNF- α levels in GBS. (g) The correlation between CSF IL-36 γ and GDSs in GBS. (h) The correlation between CSF IL-36 γ levels and CSF IL-17 levels in GBS. (i) The correlation between CSF IL-36 γ levels and CSF TNF- α levels in GBS. (j) The correlation between CSF IL-36Ra levels and GDSs in GBS. (k) The correlation between CSF IL-36Ra levels and CSF IL-17 levels in GBS. (l) The correlation between CSF IL-36Ra levels and CSF TNF- α levels in GBS.

cytokines.^[26,27] Th1 and Th17 cells play crucial roles in the pathogenesis of GBS, which suggests potential roles for IL-36 cytokines in GBS.^[28] As shown in the present study, serum IL-36 α and IL-36 γ levels in GBS were positively correlated with GDSs, while serum IL-36Ra levels were negatively correlated with GDSs. These results indicated that the increased serum levels of IL-36 α and IL-36 γ and the decreased serum IL-36Ra levels are associated with the severity of GBS. The results of correlation

analyses among IL-36 cytokines revealed negative correlations between serum IL-36 α and IL-36 γ levels with serum IL-36Ra levels in GBS, indicating that IL-36 cytokine interactions may also be one explanation for disrupted immune balance in GBS. Some representative inflammatory cytokines, such as IL-17 and TNF- α , have been confirmed to be involved in the pathogenesis of GBS.^[10,29] We further explored the correlations between the levels of IL-36 cytokines and these inflammatory cytokines in

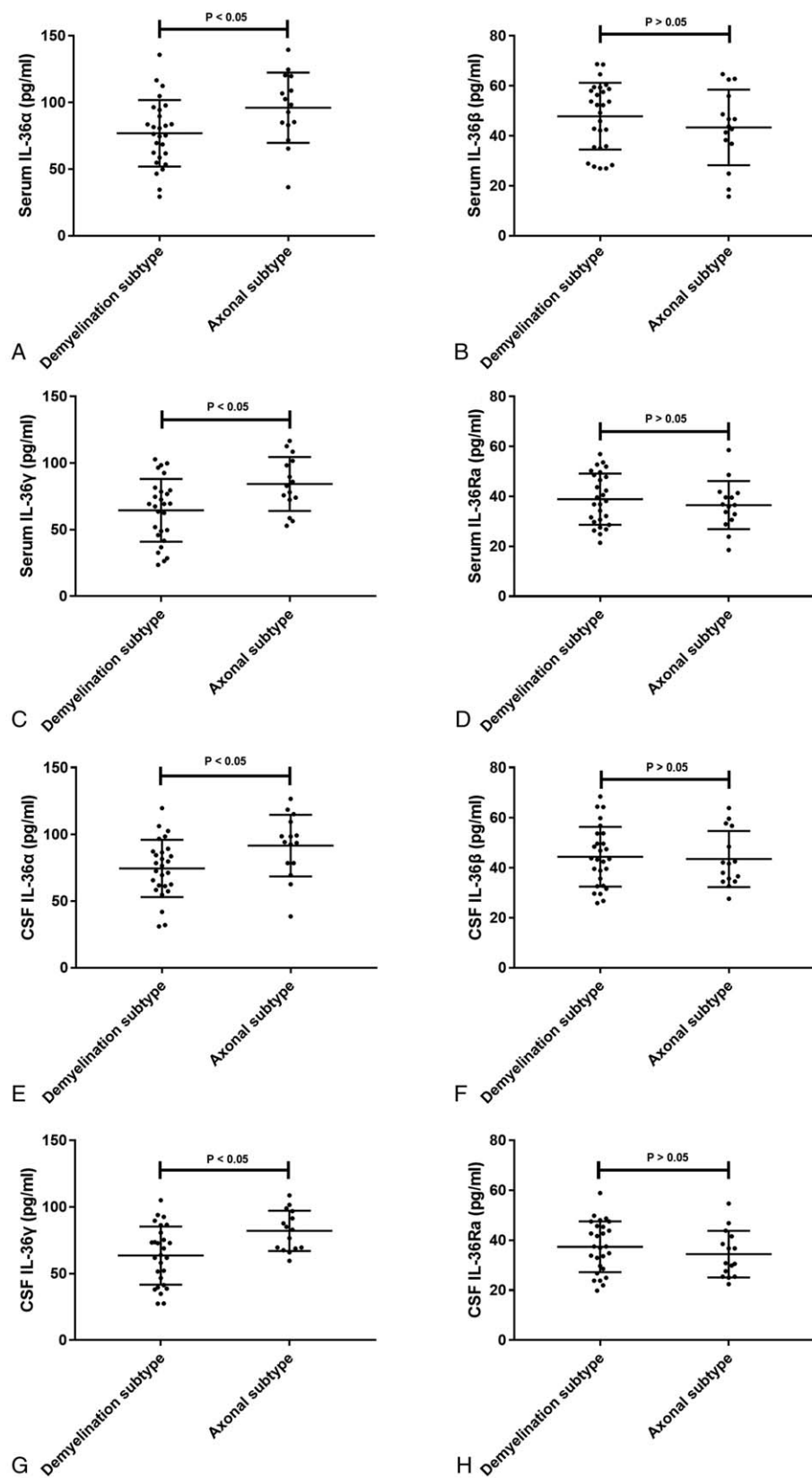


Figure 6. Serum IL-36 cytokines levels in different clinical subtypes of GBS. (a) Serum IL-36 α levels in the demyelination subtype and axonal subtype of GBS. (b) Serum IL-36 β levels in the demyelination subtype and axonal subtype of GBS. (c) Serum IL-36 γ levels in the demyelination subtype and axonal subtype of GBS. (d) Serum IL-36Ra levels in the demyelination subtype and axonal subtype of GBS. (e) CSF IL-36 α levels in the demyelination subtype and axonal subtype of GBS. (f) CSF IL-36 β levels in the demyelination subtype and axonal subtype of GBS. (g) CSF IL-36 γ levels in the demyelination subtype and axonal subtype of GBS. (h) CSF IL-36Ra levels in the demyelination subtype and axonal subtype of GBS.

GBS. Our results showed that the serum levels of IL-36 α and IL-36 γ in GBS were positively correlated with the serum levels of IL-17 and TNF- α in GBS patients, while the serum levels of IL-36Ra were negatively correlated with the serum levels of IL-17 and TNF- α . Additionally, statistically significant correlations were not observed between serum IL-36 β levels and serum IL-17 or TNF- α levels in GBS. These results and previous evidence collectively suggested that IL-36 α and IL-36 γ may aggravate inflammatory injuries in GBS patients by promoting the secretion of IL-17 and TNF- α .^[30,31] Simultaneously, IL-17 and TNF- α may also interact to induce the expression of IL-36 α and IL-36 γ in GBS. Furthermore, the secretion of IL-17 and TNF- α in GBS may be mainly affected by IL-36 α and IL-36 γ rather than IL-36 β . Recent studies have indicated that the secretion of TNF- α or IL-17 can inhibit the expression of IL-36Ra to some extent.^[30,32,33] Our results suggested that IL-36Ra expression may also be inhibited by TNF- α or IL-17 in GBS. Each individual member of IL-36 cytokines has been shown to exhibit a relatively specific expression pattern in different inflammatory diseases.^[34,35] In psoriasis and Crohn's disease, IL-36 γ expression is significantly increased and suggested to be one of the major inflammatory factors, while IL-36 β expression is not remarkably increased, suggesting a different cell source or mechanism regulating its expression.^[36] In systemic lupus erythematosus and rheumatoid arthritis, IL-36 α expression is significantly up-regulated and associated plasma cells have been identified as the main producers of IL-36 α .^[37,38] These studies and our results together suggested that IL-36 cytokines might also have distinct cell sources and expression patterns in GBS. Many pathogenic antibodies and associated immune cells play crucial roles in the occurrence and development of GBS, including plasma cells and plasmacytoid dendritic cells.^[39,40] Plasma cells, monocytes, and dendritic cells are the main producers of IL-36 cytokines, particularly IL-36 α and IL-36 γ , which may partially explain the different changes of IL-36 α , IL-36 γ , and IL-36 β in GBS.^[35,36] Additionally, similar results were observed in CSF in patients with GBS. The results of correlation analyses indicated that the CSF levels of IL-36 cytokines were also associated with the severity of GBS patients. In general, the interactions among IL-36 cytokines and other inflammatory cytokines may collectively disrupt the immune balance and aggravate inflammatory injuries in GBS patients.

Clinically, GBS is broadly divided into 2 main subtypes: the demyelination subtype and the axonal subtype. The demyelination subtype mainly refers to acute inflammatory demyelinating polyneuropathy (AIDP), which is more common in Western Europe and the United States. The axonal subtypes, consist of 2 common variants: acute motor axonal neuropathy (AMAN) and acute motor and sensory axonal neuropathy (AMSAN), which are associated with prominent axonal injuries and are relatively more prevalent in China, Japan, and Mexico.^[4,5] Our results showed that the serum and CSF levels of IL-36 α and IL-36 γ in patients with the axonal subtype of GBS were higher than those in patients with the demyelination subtype, indicating that IL-36 α and IL-36 γ may be associated with the axonal impairment in peripheral nerves and may induce more severe inflammatory reactions in GBS. The axonal subtype of GBS is closely associated with antecedent CJ infection and the lipopolysaccharides (LPS) of CJ can induce the production of anti-GM1 and anti-GQ1b antibodies in animal models.^[3,41] As shown in the study by Vigne et al, LPS can significantly stimulate the expression of IL-36 α and IL-36 γ in bone marrow-derived DCs, while the expression of IL-36 β and IL-36Ra is not remarkably increased.^[25] We postulate

that LPS from CJ may also be involved in the pathogenesis of GBS, leading to increased serum IL-36 α and IL-36 γ levels. In addition, anti-ganglioside antibodies tend to be more closely associated with the axonal subtype of GBS than the demyelination subtype, and plasma cells are the indispensable source of these antibodies.^[3,42] Simultaneously, plasma cells are also the main producers of IL-36 α and IL-36 γ , which may also explain the evaluated levels of IL-36 α and IL-36 γ in the axonal subtype of GBS.

Of course, our study still has some limitations, including the relatively small sample sizes and lack of CSF cytokine levels measured in the control groups. Further studies can include larger sample sizes, perform additional and appropriate CSF tests, or focus on the exact mechanisms of interactions among these cytokines.

In conclusion, our study is the first to explore the potential roles of IL-36 family cytokines in GBS. Our results showed that the increased serum IL-36 α and IL-36 γ levels and decreased serum IL-36Ra levels are associated with the severity of GBS. Meanwhile, the results of the correlation analyses among IL-36 cytokines and other inflammatory cytokines in GBS suggested that interactions among these cytokines are potentially involved in the pathogenesis of GBS. In addition, IL-36 α and IL-36 γ may be more closely associated with axonal injuries in GBS. Consequently, these cytokines may not only be predictors of disease severity, but also potential markers of axonal injuries in GBS.

Author contributions

Conceptualization: Zhikang Zhao, Hongbo Liu.

Data curation: Zhikang Zhao, Rui Zhang, Xinxin Gao, Hui Li.

Formal analysis: Zhikang Zhao, Hui Li.

Investigation: Zhikang Zhao, Rui Zhang, Xinxin Gao.

Supervision: Hongbo Liu.

Writing – original draft: Zhikang Zhao, Hui Li.

Writing – review & editing: Rui Zhang, Hongbo Liu.

Conceptualization: Zhikang Zhao, Hongbo Liu.

Data curation: Zhikang Zhao, Rui Zhang, Xinxin Gao, Hui Li.

Formal analysis: Zhikang Zhao, Hui Li.

Investigation: Zhikang Zhao, Rui Zhang, Xinxin Gao.

Writing – original draft: Zhikang Zhao, Hui Li.

Writing – review & editing: Hongbo Liu.

References

- van Doorn PA, Ruts L, Jacobs BC. Clinical features, pathogenesis, and treatment of Guillain-Barré syndrome. *Lancet Neurol* 2008;7:939–50.
- Leonhard SE, Mandarakas MR, Gondim F, et al. Diagnosis and management of Guillain-Barré syndrome in ten steps. *Nat Rev Neurol* 2019;15:671–83.
- Uncini A. Guillain-Barré syndrome: what have we learnt during one century? A personal historical perspective. *Rev Neurol (Paris)* 2016;172:632–44.
- Eldar AH, Chapman J. Guillain Barré syndrome and other immune mediated neuropathies: diagnosis and classification. *Autoimmun Rev* 2014;13:525–30.
- Kuwabara S, Yuki N. Axonal Guillain-Barré syndrome: concepts and controversies. *Lancet Neurol* 2013;12:1180–8.
- Loshaj-Shala A, Colzani M, Brezovska K, et al. Immunoproteomic identification of antigenic candidate *Campylobacter jejuni* and human peripheral nerve proteins involved in Guillain-Barré syndrome. *J Neuroimmunol* 2018;317:77–83.
- Rivera-Correa J, de Siqueira IC, Mota S, et al. Anti-ganglioside antibodies in patients with Zika virus infection-associated Guillain-Barré Syndrome in Brazil. *PLoS Negl Trop Dis* 2019;13:e0007695.
- Nyati KK, Prasad KN, Verma A, et al. Association of TLR4 Asp299Gly and Thr399Ile polymorphisms with Guillain-Barré syndrome in Northern Indian population. *J Neuroimmunol* 2010;218:116–9.

- [9] Goodfellow JA, Willison HJ. Guillain-Barré syndrome: a century of progress. *Nat Rev Neurol* 2016;12:723–31.
- [10] Lu MO, Zhu J. The role of cytokines in Guillain-Barré syndrome. *J Neurol* 2011;258:533–48.
- [11] Li S, Yu M, Li H, et al. IL-17 and IL-22 in cerebrospinal fluid and plasma are elevated in Guillain-Barré syndrome. *Mediators Inflamm* 2012;2012:260473.
- [12] Bassoy EY, Towne JE, Gabay C. Regulation and function of interleukin-36 cytokines. *Immunol Rev* 2018;281:169–78.
- [13] Zhou L, Todorovic V. Interleukin-36: structure, signaling and function. *Adv Exp Med Biol* 2020;20:1649.
- [14] Walsh PT, Fallon PG. The emergence of the IL-36 cytokine family as novel targets for inflammatory diseases. *Ann N Y Acad Sci* 2018;1417:23–34.
- [15] Towne JE, Garka KE, Renshaw BR, et al. Interleukin (IL)-1F6, IL-1F8, and IL-1F9 signal through IL-1Rrp2 and IL-1RAcP to activate the pathway leading to NF-kappaB and MAPKs. *J Biol Chem* 2004;279:13677–88.
- [16] Günther S, Sundberg EJ. Molecular determinants of agonist and antagonist signaling through the IL-36 receptor. *J Immunol: official journal of the American Association of Immunologists* 2014;193:921–30.
- [17] Gresnigt MS, van de Veerdonk FL. Biology of IL-36 cytokines and their role in disease. *Semin Immunol* 2013;25:458–65.
- [18] Schmitt V, Hahn M, Kästele V, et al. Interleukin-36 receptor mediates the crosstalk between plasma cells and synovial fibroblasts. *Eur J Immunol* 2017;47:2101–12.
- [19] Dinarello CA. The IL-1 family of cytokines and receptors in rheumatic diseases. *Nat Rev Rheumatol* 2019;15:612–32.
- [20] Madonna S, Girolomoni G, Dinarello CA, et al. The significance of IL-36 hyperactivation and IL-36R targeting in psoriasis. *Int J Mol Sci* 2019;20.
- [21] Wang S, Liu Y, Nie M, et al. Profile of IL-36 cytokines (IL-36 α , IL-36 β , IL-36 γ and IL-36Ra) in patients with primary immune thrombocytopenia. *Int Immunopharmacol* 2020;82:106341.
- [22] Peng J, Zhang H, Liu P, et al. IL-23 and IL-27 levels in serum are associated with the process and the recovery of Guillain-Barré syndrome. *Sci Rep* 2018;8:2824.
- [23] Queen D, Ediriweera C, Liu L. Function and regulation of IL-36 signaling in inflammatory diseases and cancer development. *Front Cell Dev Biol* 2019;7:317.
- [24] Gresnigt MS, Rösler B, Jacobs CW, et al. The IL-36 receptor pathway regulates *Aspergillus fumigatus*-induced Th1 and Th17 responses. *Eur J Immunol* 2013;43:416–26.
- [25] Vigne S, Palmer G, Lamacchia C, et al. IL-36R ligands are potent regulators of dendritic and T cells. *Blood* 2011;118:5813–23.
- [26] Mutamba S, Allison A, Mahida Y, et al. Expression of IL-1Rrp2 by human myelomonocytic cells is unique to DCs and facilitates DC maturation by IL-1F8 and IL-1F9. *Eur J Immunol* 2012;42:607–17.
- [27] Tortola L, Rosenwald E, Abel B, et al. Psoriasiform dermatitis is driven by IL-36-mediated DC-keratinocyte crosstalk. *J Clin Invest* 2012;122:3965–76.
- [28] Chen X, Guo Y, Han R, et al. Class I PI3K inhibitor ZSTK474 attenuates experimental autoimmune neuritis by decreasing the frequency of Th1/Th17 cells and reducing the production of proinflammatory cytokines. *Cell Immunol* 2018;329:41–9.
- [29] Han R, Xiao J, Zhai H, et al. Dimethyl fumarate attenuates experimental autoimmune neuritis through the nuclear factor erythroid-derived 2-related factor 2/hemoxygenase-1 pathway by altering the balance of M1/M2 macrophages. *J Neuroinflammation* 2016;13:97.
- [30] Furue K, Yamamura K, Tsuji G, et al. Highlighting interleukin-36 signalling in plaque psoriasis and pustular psoriasis. *Acta Derm Venereol* 2018;98:5–13.
- [31] Żebrowska A, Woźniacka A, Juczyńska K, et al. Correlation between IL36 α and IL17 and activity of the disease in selected autoimmune blistering diseases. *Mediators Inflamm* 2017;2017:8980534.
- [32] Do MS, Jeong HS, Choi BH, et al. Inflammatory gene expression patterns revealed by DNA microarray analysis in TNF-alpha-treated SGBS human adipocytes. *Yonsei Med J* 2006;47:729–36.
- [33] Mercurio L, Morelli M, Scarponi C, et al. IL-38 has an anti-inflammatory action in psoriasis and its expression correlates with disease severity and therapeutic response to anti-IL-17A treatment. *Cell Death Dis* 2018;9:1104.
- [34] Murrieta-Coxca JM, Rodríguez-Martínez S, Cancino-Díaz ME, et al. IL-36 cytokines: regulators of inflammatory responses and their emerging role in immunology of reproduction. *Int J Mol Sci* 2019;20:1649.
- [35] Buhl AL, Wenzel J. Interleukin-36 in infectious and inflammatory skin diseases. *Front Immunol* 2019;10:1162.
- [36] Boutet MA, Bart G, Penhoat M, et al. Distinct expression of interleukin (IL)-36 α , β and γ , their antagonist IL-36Ra and IL-38 in psoriasis, rheumatoid arthritis and Crohn's disease. *Clin Exp Immunol* 2016;184:159–73.
- [37] Chu M, Wong CK, Cai Z, et al. Elevated expression and pro-inflammatory activity of IL-36 in patients with systemic lupus erythematosus. *Molecules* 2015;20:19588–604.
- [38] Frey S, Derer A, Messbacher ME, et al. The novel cytokine interleukin-36 α is expressed in psoriatic and rheumatoid arthritis synovium. *Ann Rheum Dis* 2013;72:1569–74.
- [39] Naik GS, Meena AK, Reddy B, et al. Anti-ganglioside antibodies profile in Guillain-Barré syndrome: correlation with clinical features, electrophysiological pattern, and outcome. *Neurol India* 2017;65:1001–5.
- [40] Wang YZ, Feng XG, Wang Q, et al. Increased plasmacytoid dendritic cells in Guillain-Barré syndrome. *J Neuroimmunol* 2015;283:1–6.
- [41] Ang CW, De Klerk MA, Endtz HP, et al. Guillain-Barré syndrome- and Miller Fisher syndrome-associated *Campylobacter jejuni* lipopolysaccharides induce anti-GM1 and anti-GQ1b antibodies in rabbits. *Infect Immun* 2001;69:2462–9.
- [42] Nutt SL, Hodgkin PD, Tarlinton DM, et al. The generation of antibody-secreting plasma cells. *Nat Rev Immunol* 2015;15:160–71.