The Immuno-Inflammatory Effect of IL-27 in Preterm Labor

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Research Article

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Abstract

Objective

To investigate the effect of IL-27 on Th1 cells infiltration in human fetal membranes (FMs) and the underlying mechanisms in preterm labor.

Methods

The expression of IL-27 receptor α subunit (IL-27R α) and Th1 cells were studied in human FMs from pregnant women with preterm labor (PL) and term labor (TL). *In vitro*, we investigated the effects of IL-27 in human amniotic cell lineage on the Th1 related chemokines and adhesive molecules and the underlying intracellular signaling molecules.

Results

The expression of IL-27R α was higher in human fetal membranes from PL group compared with TL group. Compared with TL group, the PL group had more Th1 cells infiltration in human FMs. Meanwhile, it was confirmed the expression of CXCL9, CXCL10, CXCL11 and ICAM-1 were higher in FMs from PL group to TL group. Moreover, IL-27 stimulation can up-regulate the expression of those chemokines in human amniotic cell lineage via JAK2/STAT1/STAT3 signaling pathway.

Conclusions

The expression of IL-27R α and number of Th1 cells infiltration were higher at FMs of PL group than TL group. IL-27 could promote Th1 related chemokines and adhesive molecules in human amniotic cell lineage mainly through JAK2/STAT1/STAT3 signaling pathway.

Introduction

Preterm labor (PL) accounts 5 to 18% of pregnancies and is a leading cause of infant morbidity and mortality[1]. Apart from intra-amniotic inflammation, maternal-fetal immune imbalance is also an etiology of PL. A wealth of evidence showed that T cells played an important role in pregnancy[2–4]. As one subgroup of CD4⁺ T cells, Th1 cells, secreting proinflammatory cytokines such as IFN- γ , IL-2 and TNF- α , are reported to be involved in the initiation and maintenance of inflammatory process[2]. Th1-type immunity was incompatible with successful pregnancy[3], such as miscarriages[4], preeclampsia[5] or infertile polycystic ovary syndrome[6] in peripheral blood. And increased percentages of IFN- γ +Th1 cells were found in the decidua of women who miscarried a genetically normal fetus compared with those of induced abortions women[7]. Also, the number of Th1 cells and Th1/Th2 ratio were higher in decidua of unexplained recurrent spontaneous abortion patients compared with controls[8]. As for PL, there were significantly higher levels of Th1 cytokines in peripheral blood from women of premature rupture of membranes[9] and PL[10]. In 2007, Curry performed a case-control study with 101,042 pregnancies, indicating that Th1 cells-inducing cytokines—IFN- γ > 90th in peripheral blood was associated with an increased risk of delivering at 30–33 weeks, while IFN- γ > 75th percentile was associated with an increased risk of delivering at 34–36 weeks[11]. At the maternal-fetal interface, higher Th1 cells-inducing cytokines were found in cervicovaginal fluid[12] and placentas[13] in women with PL.

Interleukin-27 (IL-27), a member of IL-6/IL-12 family, could promote Th1 polarization and infiltration. Yoshida stated that IL-27 could stimulate IFN- γ secretion in response to primary stimulation of Leishmania *in vitro*. IL-27R α -/- mice show impaired IFN- γ production early in the infection of Leishmania[14]. Intranasal injection of IL-27 in mice models of ovalbumin-induced asthma could upregulate the type 1 T helper (Th1)-T memory (Tm) cell subsets of lung tissue lymphocytes, and reduce airway inflammation and hyperresponsiveness[15]. Meanwhile, IL-27 could upregulate the expression of chemokines such as C-C motif ligand (CCL) 2, C-X-C motif chemokine ligand (CXCL)9, CXCL10, CXCL11 and adhesive molecules such as lymphocyte function-associated antigen1, intercellular adhesion molecule-1 (ICAM-1)[15–20], recruiting Th1 cells or other immune cells.

As we know, human FMs are composed of epithelial cells, interstitial fibrous layer, chorionic layer and parietal decidual layer. Since it is between the mother and the fetus, it could be an ideal tissue for studying the specific immune tolerance. In addition, abundant leukocytes infiltrate at the mother and fetal interface in PL[21, 22], including the participation of Th1 cells. Based on this, we speculate that IL-27 may mediate PL by promoting the infiltration of Th1 cells in FMs.

Materials And Methods

Human samples

16 pregnant women with singleton pregnancies from 28 to 36+6 weeks from the first affiliated hospital of Chongqing medical university were enrolled and divided into PL group and TL group with informed written consent. PL and TL were defined according to the guidelines of the American College of Obstetricians and Gynecologists (ACOG). Pregnant women who suffered preterm labor with infection[23] and pregnancy complications such as pregnancy hypertension, diabetes, placental abruption or chronic diseases were excluded. The basic characteristic of these patients was presented in Table 1. Human FMs were collected within 30 minutes after delivery. These samples were stored as required for Western blot, real-time quantitative PCR (qPCR) and Immunohistochemistry. Ethics approval was gained from the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (2019-137).

Cell culture

Human fetal membranes could secrete chemokines recruiting T cells[24]. Therefore, human amniotic cell lineage was used as the targeting cells in the present study. Human amniotic cell lineage—WISH (Wistar Institute Susan Hayflick) cell line was purchased from the commercialized company. They were cultivated in MEM (Gibco, USA) with 10% fetal bovine serum (PAN, Germany), 1% nonessential Amino Acid Solution, 100µg/ml streptomycin and 100 U/ml penicillin (Beyotime Biotechnology, Shanghai, China). Cells were kept in a wetted 5% CO₂ atmosphere at 37 °C and the medium was changed every two days.

Extraction of total RNA and qPCR test

Total RNA was extracted by RNAiso Plus (Takara Bio Inc., Tokyo, Japan) and quantified by measuring the ratio of A 260nm/A 280nm. Total RNA was reverse transcribed using a PrimeScript RT Reagent Kit (Takara Bio Inc., Tokyo, Japan). Thereafter, generated cDNA was subjected to qPCR analysis using SYBR Premix Ex Taq II kit (MCE, Shanghai, China) with specific primers (Table 2). Amplification and detection of mRNA was performed using Thermal Cycler Dice Real Time System (TP800; Takara Bio, Shiga, Japan). Relative quantity of target gene expression to β -actin gene was measured by comparative Ct method as described previously.

Western Blot

Total protein was harvested from the tissue by RIPA lysis buffer (ZSGB-BIO, Beijing, China) containing PMSF (99:1). Equal amounts of protein (40 µg) were electrophoresed on 10% SDS-polyacrylamide gels (Invitrogen) and blotted onto PVDF membranes. The membranes were incubated overnight with primary antibodies (IL-27R α (1:1000; Affinity Biosciences, USA); Janus kinase 2 (JAK2), P-JAK2(Tyr1007, (1:1000; Affinity Biosciences, USA)), signal transducer and activator of transcription 1 (STAT1), p-STAT (Tyr701, (1:1000; Affinity Biosciences, USA)), STAT3, p-STAT3 (Tyr705, (1:1000; Affinity Biosciences, USA)) after blocked in 5% nonfat milk for 2 h; Then, the membranes were incubated with an HRP-conjugated anti-IgG secondary antibody, followed by detection with an ECL chemiluminescent detection system. The blots were imaged and quantified using ImageJ software, and the results were reported as primary antibody/ β -actin ratio.

Hematoxylin and eosin (H& E) staining and immunohistochemistry (IHC)

Fetal membrane tissues were formalin fixed, and paraffin-embedded sections were stained with hematoxylin and eosin. IHC was used to localize IL-27R α + and T-bet+ cells within the fetal membranes. Paraffin sections of human FMs were dewaxed and rehydrated, followed by microwave antigen retrieval. Then, these sections were blocked with 3% H2O2 followed by 10% normal goat serum. Following that, these tissue sections were incubated with primary antibodies overnight at 4 centigrade (IL-27R α , (1:200, santa, China); T-bet, (1:300, protein tech, China)). After thorough washes, they were incubated with biotinylated goat anti-mouse/Rabbit IgG. Positive antibody binding was detected with diaminobenzidine, followed by staining with hemotoxylin (Sigma).

ELISA Assay

Supernatant of cells culture were harvested and stored at -80°C until analyzed by Elisa Assay. Frozen human FMs were thawed on ice and solubilized in ice-cold PBS containing protease inhibitors PMSF. Samples were centrifuged at 5,000 g for 10 min at 4°C, and supernatant was stored at -80°C until analyzed by Elisa Assay. The total protein of human FMs homogenates was determined using Bradford's reagent (Bio-Rad Protein Assay; Bio-Rad) according to the manufacturer's instructions. Both the supernatant of cells culture and FMs tissue homogenate were measured by a human CCL2, CXCL9, CXCL10, and CXCL11 Elisa kit (Jiubang, China). All the experiments were conducted according to the manufacturer's instructions. The absorbance of the supernatant was assessed at 450nm in a Multi-skan GO plate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

Statistical analysis was performed by prism soft-ware. Student's t test or Mann–Whitney U test were used to assess continuous variables according to its distribution. Chi-square test were used to assess categorical variables. A p value< 0.05 was considered statistically significant.

Results

1. Expression of IL-27R α in Human FMs in PTLI and TL Group

Our previous team work had proved that serum IL-27 level was higher in the PL group than women in the TL group[25]. In the present study, qPCR test (Fig. 1a) and western blot analysis (Fig. 1b and 1c) confirmed that the expression of IL-27R α in FMs was significantly higher in PL group than TL group. Our H& E staining had showed the structure of human FMs, which mainly consisted of amnion layer, interstitial fibrous layer, chorion layer and decidua parietalis layer (Fig. 1d). In the immunostaining tissue sections, IL-27R α was expressed in amnion cells, chorion cells and decidua cells (Fig. 1e and Fig. 1f).

2. Th1 (T-bet+) Cells Infiltration at the Maternal-fetal Interface of Human

The expression of four main CD4 + T cells subgroups' transcription factors (T-bet-Th1, GATA3-Th2, ROR γ t-Th17, FOXP3-Treg) were analyzed by qPCR in human FMs. As a result, T-bet mRNA expression was mostly elevated in PL group compared with that in TL group(Fig. 2a). Then, T-bet expression was analyzed by Western blot analysis and IHC in human FMs. As a result, T-bet protein expression was higher in FMs from PL group than those in TL group (Fig. 2b and Fig. 2c). In the immunostaining tissue sections, the major location of Th1 cells was decidua parietalis layer and chorion layer (Fig. 2d and Fig. 2e).

3. The Expression of Inflammatory Mediators were Higher at FMs from PL Group than TL Group

The expression of CXCL9, CXCL10, CXCL11 and ICAM-1 were compared in human FMs between PL and TL groups. The mRNA expression of CXCL9, CXCL10, CXCL11, and ICAM-1 were higher at human FMs of PL group than TL group. Furthermore, the protein levels of CXCL9, CXCL10, CXCL11 in the human FMs

homogenate in PL group were also higher compared with those in TL group. In addition, Weston blot results showed that the protein expression of ICAM-1 was higher in PL group than TL group.

4. IL-27 Could Upregulate the Expression of Th1 cells Related Chemokines and Adhesive Molecules in WISH cells

As T cells were mainly attracted to the maternal-fetal interface by chemokines and adhesive molecules, we analyzed Th1 cells related chemokines—CXCL9, CXCL10, CXCL11 and ICAM1 in WISH cells stimulated by IL-27 or not. The mRNA expression of CXCL9, CXCL10, CXCL11 and ICAM1 were analyzed after IL-27 treatment for 6, 12 or 24 h at different dose (10 ng/mL and 50 ng/mL). As a result, IL-27 could significantly upregulate the mRNA expression of CXCL9, CXCL10, CXCL11, mainly peaking at 12 hours (Fig. 4a and Fig. 4b). And this upregulation was dose dependent, peaking at 50 ng/mL IL-27 (Fig. 4c). Therefore, we treated WISH cells with 50 ng/mL IL-27 for further analysis. The Elisa assay confirmed that upregulation of CXCL9, CXCL10, CXCL11 protein levels in the supernatant of WISH cells (Fig. 4d,e,f), which were consistent with qPCR analysis. Weston blot analysis also showed that IL-27 could upregulate ICAM-1 expression in WISH cells (Fig. 5e).

5. The Role of IL-27 in the Activation of the JAK2/STAT1/STAT3 Signaling Pathways at Fetal Membranes

As JAK/STAT signaling pathway played a critical role in Th1 related reactions, we investigate if IL-27 could upregulate its expression in WISH cells. IL-27 was added at 10, 20, 30, 60, 120, 240 min in the culture medium of WISH cells, and total protein and phosphate protein of JAK2, STAT1, and STAT3 were investigated by Weston blot analysis (Fig. 5a). As a result, phosphorylated JAK2 was peaked at 10 minutes and then weakened gradually. As time accumulated, phosphorylated STAT1 / STAT3 increased sequentially. To further investigate the role of JAK/STAT signaling pathway, JAK2 inhibitor-AG490 (10uM) was added in WISH cells one hour before IL-27 was added. The data of qPCR (Fig. 5b), Elisa (Fig. 5c) and Weston blot (Fig. 5d and 5e) revealed that AG490 could effectively inhibit the expression of CXCL9, CXCL10, CXCL11, however, except for ICAM-1.

Discussion

The immune tolerance of human FM plays an important role in maintaining pregnancy, and excessive inflammation is one of the mechanism leading to PL [26]. A wealth of evidence had demonstrated that Th1 cells were associated with PL by secreting proinflammatory cytokines such as IFN- γ and TNF- α [2]. However, there are few literatures to study the underlying mechanism of Th1 cells infiltration in FMs of PL. According to our previous data, we hypothesized that IL-27 can mediate Th1 cells infiltration at human FM involving in the pathological mechanism of PL.

As a well-known specific transcription factor of Th1 cells, T-bet regulates the differentiation and development of Th1 cells. Thus, we measured the expression of T-bet to represent Th1 cells, as previous literature reported [26, 27]. The results showed that Th1 cells expression at FMs from PL group were more than those from TL group, suggesting that Th1 cells may related with PL, which was consistent with previous studies [28]. And the immunohistochemistry analysis revealed that Th1 cells were mainly located in partial decidua and chorion layer, similar with the findings of other literature [29]. Studies have shown that the leukocytes residing at FM could be maternal origin [30], and the blood vessels in the chorionic and decidual layers may be the main source of these infiltrating cells.

Next, we are thinking about what factors can regulate the changes of Th1 cells at the maternal-fetal interface? It is well known that the migration of Th1 cells is related to CXCL9, CXCL10 and CXCL11, which belong to the CXC chemokine family. In addition, the chemotactic effect of leukocytes often depends on ICAM-1, which is recognized to regulate rolling and stagnation [31]. Previous studies have shown that IL-27 can significantly up-regulate the expression of CXCL9 and CXCL10 in human oral epithelial cells [32] and rheumatic arthritis fibroblast-like synovial cells in vitro [33], and enhance the expression of CXCL9, CXCL10 and CXCL11 in mouse models of psoriasis-like skin diseases [33] and Con A-induced hepatitis [34] in vivo. At the same time, the up-regulation of ICAM-1 was also found in human coronary artery endothelial cells [35] and fibroblast-like synovial cells [19] stimulated by IL-27. Therefore, we speculate whether the changes of Th1 cells at the maternal-fetal interface are related to the regulation of IL-27? Indeed, our results found that IL-27 can up-regulate the expression of CXCL9, CXCL10, CXCL11 and ICAM-1 in WISH cells. A previous study had confirmed that the expression of CXCL9, CXCL10 and CXCL11 mRNA in chorion-amniotic membrane of chronic chorioamnionitis was higher than the control group [36]. Meanwhile, it was also confirmed that IL-27 can up-regulate the expression of ICAM-1 in WISH cells. This was in consistent with a study of 622 pregnant women with threatened preterm birth, ICAM-1 was found to be significantly associated with PL [37]. Therefore, these results help us better understand the reason why Th1 cells accumulated at the maternal-fetal interface in PL. It also should be noted that infiltrative CD4 + T cells could also promote the local production of CXCL9 and CXCL10 [38]. Thus, there was a positive feedback loop in this process.

Then, what is the specific molecular mechanism of IL-27 regulating these chemotactic mediators? In our study, IL-27 could up-regulate the expression of CXCL9, CXCL10, CXCL11 in WISH cells by activating the JAK/STAT signaling pathway. This was similar with the conclusions in the previous studies. IL-27 could up-regulate the expression of CXCL9, CXCL10 and CXCL11 in human hepatocytes through STAT1 [34] and enhance the expression of CXCL10 in human keratinocytes by STAT1 and STAT3 [39]. Furthermore, AG490 (JAK2 inhibitor) could also inhibit the expression of SOCS1 in activated CD4 + T cells from cord blood mononuclear cells [40] and ABCA1 expression in macrophages [41] stimulated by IL-27.

In this experiment, we first tried to explore the regulatory relationship between IL-27 and Th1 cells at the maternal-fetal interface, in order to partly explain the reason for Th1 cell infiltration in PL. However, it still has certain limitations. For example, these T-bet positive cells cannot be completely interpreted as Th1 cells, and their biological functions and mechanisms need further studies. Secondly, it is worth noting that the difference in Th1 cells in the maternal-fetal interface cannot be completely explained by the difference in IL-27 expression. It may be affected by many factors such as gestational age. Meanwhile, it should be noted that the number of Th1 cells in chorion layer was relatively small, further studies are needed to determine their contribution to PL.

Conclusions

In conclusion, our research shows that IL-27 may play a role in the pathogenesis of PL by regulating the infiltration of Th1 cells in FM. This effect is mainly achieved by activating the JAK2/STAT1/STAT3 signaling pathway to up-regulate the expression of CCL2, CXCL9, CXCL10 and CXCL11. Based on the maternal-fetal interface, this research provides new insights into the regulation of Th1 cell infiltration by IL-27 and further enriches the pathogenesis theory of PL.

Abbreviations

FMs, fetal membranes; IL-27Ra, IL-27 receptor α subunit; PL, preterm labor; TL, term labor; Th1 cells, type 1 helper cells; PROM, premature rupture of membranes; CCL, C-C motif ligand; CXCL, C-X-C motif chemokine ligand; ICAM-1, intercellular adhesion molecule-1; JAK, Janus kinase 2; STAT, signal transducer and activator of transcription.

Declarations

Ethics approval and consent to participate

Ethics approval was gained from the Ethics Committee of the First Affiliated Hospital of Chongqing Medical University (2019-137), and Written informed consent for participation was obtained from all participants.

Consent for publication

Written informed consent for participation was obtained from the participants.

Availability of data and materials

Data and materials would be provided if requested

Competing interests

There were no competing interests to declare

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Author contributions

Youwen Mei designed the study, performed the experiments, analyzed the data, and wrote the manuscript. Nanlin Yin and Hongbo Qi designed the study, reviewed the manuscript, organized financial support, and supervised the project.

Yuxin Ran, Zheng Liu, Yunqian Zhou and Jie He performed the experiments, and analyzed the data. All authors approved the submitted version of the manuscript.

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Tables

Table 1
Characteristics of pregnant women from TL and PT groups

Characteristic	Maternal age (Mean $\pm$ SD)	BMI before pregnant (Mean $\pm$ SD)	BMI of pregnant (Mean $\pm$ SD)	Gravity times(n)	Parity times(n)	GA (weeks)	Total leucocyte count(*10 ⁹ /L, Mean $\pm$ SD)	Neutrophil count (Mean $\pm$ SD)	Percentage of neutrophils (Mean $\pm$ SD)	lymphocyte count (Mean $\pm$ SD)	Perce of lymph (Mean $\pm$ SD)
TL(n = 8)	28.88 $\pm$ 1.64	20.25 $\pm$ 2.13	26.09 $\pm$ 2.34	2(1–2)	0(0–1)	38.86 $\pm$ 0.4	10.19 $\pm$ 1.66	8.2 $\pm$ 1.54	80.24 $\pm$ 2.6	1.28 $\pm$ 0.18	12.73 $\pm$ 1.84
PTL(n = 8)	29.25 $\pm$ 3.58	21.8 $\pm$ 3.2	26.38 $\pm$ 3.47	2.5(1.25–3)	0(0–0.75)	34.86 $\pm$ 1.31	12.46 $\pm$ 1.3	9.93 $\pm$ 1.69	79.34 $\pm$ 6.11	1.74 $\pm$ 0.47	14.29 $\pm$ 4.55
P	0.791	0.277	0.85	0.161	0.721	< 0.0001	0.009	0.051	0.71	0.031	0.391

Table 2
Primers used for qPCR

Human	Sense sequence	Anti-sense sequence
IL-27	CCTGGTTCAAGCTGGTGTCT	CTCCTGGCAGGTGAGATTCC
IL-27Ra	CTGGAACCGGAGCTGAAGAC	CGCCCCACAAATCCTCTTCT
T-BET	CAACGCTTCCAACACGCATA	GTAATCTCGGCATTCTGGTAGG
GATA3	TCTGACCGAGCAGGTCGTA	GGCGACGACTCTGCAATTCT
ROR γ t	GGCACCCTACCCTTTACCTG	TCTTGGCCTTCATTGTACCCT
Foxp3	ACACCGTACAGCGTGGTTTT	TGGACTGACAGAAAAGGATCAGC
CXCL9	GTTCTGATTGGAGTGCAAGGA	ATGATTTCAATTTCTCGCAGG
CXCL10	TCTCCCATCACTTCCTACAT	GGAGTAGTAGCAGCTGATTTGG
CXCL11	GTGAAGGGCATGGCTATAGC	TTGTTACTTGGGTACATTATGGAGG
ICAM-1	GAACCCATTGCCCGAGCTCA	TGACAGTCACTGATTCCCCGAT
β -ACTIN	TGGCACCCAGCACAAATGAA	CTAAGTCATAGTCCGCCTAGAAGCA

Figures

Figure 1

Analysis of IL-27Ra expression in human FMs. (a) IL-27Ra mRNA expression in PL and TL group. (b) IL-27Ra protein expression in PL and TL group. The first three are representative of samples of TL group and the last three are from the PL group. (c) Quantitatively analyzed band intensities of IL-27Ra were normalized against β-actin. (d) H&E staining of fetal membranes. (e) Immunohistochemical staining for IL-27Ra in human FM from TL group and (d) PL group. AE, amnion epithelium; CL, connective tissue layer; CT, chorionic trophoblast layer; DEC, decidua. Scale bar (d-f) 400 μm. * $p < 0.05$ ($n = 6-8$)

Figure 2

Analysis of T-bet expression in human FMs. (a) The mRNA expression of four main CD4⁺ T cells subgroups' transcription factors (T-bet-Th1, GATA3-Th2, RORγ-Th17, FOXP3-Treg) in human FMs from PL and TL group. (b) T-bet protein expression in PL and TL group. The first three are representative of samples

of TL group and the last three are from the PL group. (c) Quantitatively analyzed band intensities of T-bet were normalized against β -actin. (d) Immunohistochemical staining of T-bet in human FMs from TL group and (e) PL group. Scale bar (d-e) 400 μ m. * p < 0.05 (n = 6-8).

Figure 3

The comparison of chemokines and ICAM-1 in FMs from PL and TL group. (a) Comparison of CXCL9, CXCL10, CXCL11, and ICAM-1 mRNA expression in PL and TL group. (b) Comparison of CXCL9, CXCL10, CXCL11 protein expression in FMs homogenate from PL and TL group. (c) ICAM1 protein expression in PL and TL group. The first three are representative of samples of TL group and the last three are from the PL group. (c) Quantitatively analyzed band intensities of T-bet were normalized against β -actin. * p < 0.05, ** p < 0.01, and *** p < 0.001 (n = 6-8).

Figure 4

Analysis of chemokines and ICAM-1 stimulated by IL-27 in WISH cells. (a) The effect of 10 ng/mL IL-27 and 50ng/mL IL-27 (b) on the mRNA expression of CXCL9, CXCL10, CXCL11 and ICAM-1. (c) Dose dependent effect of IL-27 on the upregulation of CXCL9, CXCL10, CXCL11 and ICAM-1 mRNA expression. (d) The effect of 10 ng/mL IL-27 and 50ng/mL IL-27 (e) on the protein expression of CXCL9, CXCL10, CXCL11 and ICAM-1 in the supernatant of WISH cells by

Elisa assay. (f) Dose dependent effect of IL-27 on the upregulation of CXCL9, CXCL10, CXCL11 and ICAM-1 protein levels in the supernatant of WISH cells by Elisa assay.*p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001 (n=3).

Figure 5

IL-27 induces the upregulation of CXCL9, CXCL10, CXCL11 and ICAM-1 in WISH cells through JAK2/STAT1/STAT3 pathway. (a) WISH cells were stimulated with IL-27 (50 ng/mL) for 0,10,20,30,60,120 minutes. Total protein and phosphorylated JAK2, STAT1, and STAT3 protein expression were analyzed by western blot analysis. (b) The effect of JAK2 inhibitor-AG490 on the mRNA expression of CXCL9, CXCL10, CXCL11 and ICAM-1 in WISH cells stimulated by IL-27 by qPCR analysis. (c) The effect of AG490 on the protein expression of CXCL9, CXCL10, CXCL11 and ICAM-1 in the supernatant of WISH cells stimulated by IL-27 by Elisa assay. (d) The effect of IL-27 and AG490 on the protein expression of ICAM-1 in WISH cells stimulated by IL-27 by Weston blot analysis. (e) Quantitatively analyzed band intensities of ICAM-1 were normalized against β -actin. *p < 0.05, **p < 0.01, and ***p < 0.001 (n = 3)

Supplementary Files

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- [SupTable1.doc](#)