The higher expression of monocyte chemotactic protein 1 in mild COVID-19 patients is correlation with the inhibition of IFN signaling

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Abstract

Background: The level of chemokine was markedly elevated in severe COVID-19 patients. But the role of chemokine in mild COVID-19 has not yet been established. However, most of the COVID-19 patients in Shiyan City, China, had mild cases. The purpose of this study is to evaluate the level of chemokine in mild COVID-19 patients and to explore the correlation between chemokine and host immune response.

Methods: In this study, the level of chemokine in the serum for COVID-19 patients in Shiyan City was detected by ELISA. The expression of chemokine receptor and other signal molecular was detected by real-time PCR.

Results: We first demonstrated that COVID-19 patients are characterized by higher levels of chemokine. Meanwhile, monocyte chemotactic protein 1 (MCP-1) has shown higher expression in patients with mild cases of COVID-19. The receptor of MCP-1, CCR2 was also found to be expressed at higher levels in the same mild COVID-19 patients. Finally, the higher expression of MCP-1 in mild COVID-19 patients is correlated with the inhibition of IFN signaling.

Conclusion: These findings add to our understanding of the immune-pathologic mechanisms of SARS-CoV-2 infection, and provide potential therapeutic targets and strategies. MCP-1 may be an effective indicator in mild patients, and early use of interferon has a good antiviral therapeutic effect.

Background

An outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spread throughout the world[1, 2]. Globally, by September 2020, there have been 31,425,029 confirmed cases of COVID-19, including 967,164 deaths (https://covid19.who.int/). According to the epidemiological statistics, most of the COVID-19 (about 80%) belong to the mild[3, 4]. Although the city of Shiyan is located in Hubei Province, most of the COVID-19 patients there had mild cases[4]. For this reason, it is necessary to study the immunological characteristics of mild COVID-19 patients and find a suitable therapeutic strategy on the basis.

Immune response is the body's defense mechanism against viral infection. It involves the innate and adaptive immune responses. However, excessive immune responses after infection, also called a cytokine storms, have been found to be associated with extreme levels of pro-inflammatory cytokines. Patients infected with COVID-19 showed higher leukocyte numbers, abnormal respiratory findings, and increased level of plasma pro-inflammatory cytokines. The direct cause of death from acute COVID-19 involves cytokine storm that damage to lungs and multiple organs of the body, leading to multiple organ exhaustion[5].

Preliminary studies have shown that SARS-CoV-2 infection may triggers a cytokine storm, and results in the increase of a variety of cytokines, including chemokine[5-7]. Chemokines are low-molecular-weight proteins with powerful chemo-attractant activity. They play a role in the immune cell recruitment during

inflammation. Chemokines are classified according to their chemical structure, the C, CC CXC and CX3C families[8]. The binding of chemokines to their receptors is responsible for their chemo-attractant ability. The chemokine receptors are seven-transmembrane-spanning, G-protein-coupled receptors. They are expressed on leukocytes and endothelial cells, etc.[9]. Serum chemokine levels were found to be elevated in patients with COVID-19, and they were even higher in those who required ICU admission, suggesting a relationship with lung damage and disease severity[10]. However, it is not clear whether the concentration of chemokine is elevated in mild COVID-19 patients, and the highly expressed chemokine can be used as a marker of the diagnosis and prognosis of mild COVID-19.

Type I interferon (IFNs) (including IFN-α and IFN-β) have broad-spectrum antiviral activities, which act by inducing an antiviral response and mediating adaptive immune response[11]. Infection of cells with virus causes the activation of several cellular transcription factors, such as interferon regulatory factor 3/7 (IRF3/7) and NF-κB, which activate the expression of a number of interferon-stimulated genes (ISGs) and exert antiviral effect[12,13]. The activation of transcription factors also induce the secretion of chemokine, which further recruit and coordinate specific subsets of leukocytes, such as neutrophils and monocytes[14,15]. This means that the secretion of chemokine may be related to the release of interferon, but the specific relationship between them in the process of SARS-CoV-2 infection has not been clearly established.

Clinically, Type I IFNs have already been approved for use in the treatment of certain cancers, autoimmune disorders, and viral infections[16]. Type I IFNs are currently in clinical trials to evaluate their ability to treat MERS-CoV[17] and therefore have been proposed for the treatment of COVID-19, but there is currently no evidence from laboratory testing against SARS-CoV-2.

In this study, the concentration of chemokine in serum and the expression level of chemokine receptor in PBMCs from COVID-19 patients were detected, so as to evaluate the role of chemokine in mild COVID-19 in Shiyan City, China. Then we detected the level of IFN-β and the relationship between IRF3 and chemokine, so as to prove the correlation between chemokine and interferon signal. Our results suggested that the higher expression of monocyte chemotactic protein 1 (MCP-1) in mild COVID-19 patients is correlation with the inhibition of IFN signaling.

Methods

Subjects.

The diagnose of COVID-19 was graded according to China National Health Commission Guidelines for Diagnosis and Treatment of SARS-CoV-2 infection (seventh version). 10 severe and 30 mild COVID-19 patients were recruited from Xiyuan Hospital and Renmin Hospital, Shiyan City and the study protocol received approval from the Clinical Ethics Committee of Hubei University of Medicine (2020-TH-017). Another 10 healthy subjects were recruited as a control group. All the healthy subjects were not

complicated with tumor, infection or other diseases. All individuals gave their informed consent to participate. The basic information of COVID-19 patients and healthy controls is listed in Table 1.

Isolation of Peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated through density-gradient centrifugation using Ficoll-Paque (Sigma, 17144002) from peripheral blood samples of the participants in this study. 1×10^6 PBMCs were isolated from each milliliter of peripheral blood. Isolated 3×10^6 PBMCs were reverse transcribed into cDNA directly.

Real-time PCR

After RNA was isolated from PBMCs, it was reverse transcribed into cDNA. Real-time quantitative PCR was performed to quantify chemokine receptors and transcript factor and GAPDH levels by using SYBR Premix Ex Taq (TaKaRa, RR820A) in the Bio-rad CFX manager 3.1 software. The comparative Ct method was used to calculate the relative abundance of difference genes as compared with the expression of GAPDH. The primer sequences were listed in Table 2.

Enzyme linked immunosorbent assay (ELISA)

The chemokine MCP-1, interferon-inducible protein-10 (IP-10), Interleukin 8 (IL-8) and interferon β (IFN-β) in the serum of COVID-19 patients and healthy controls were determined by ELISA kit according to the manufacturer's instruction (1117392, 1110802, 1117452, Dakewei.Inc, KE00187, Sanying. Inc.). The undiluted serum samples were added to the pre-coated ELISA plate, incubated for 2h, and enzyme labeled antibody was then added. After washing the plate for 5 times, the substrate was added for color development, The absorbance was then determined using Epoch Microplate Spectrophotometer (Bio-Tek Instruments, Inc.) at 490 nm.

Statistical analysis

Analysis of Variance (ANOVA) tests were used to compare the plasma chemokine levels among the COVID-19 patients and healthy control groups. The Spearman rank correlation coefficient was used for linear correlation analysis between the expression level of plasma chemokine and IRF3. All data were analyzed using SPSS version 19.0 and GraphPad 5.0 software. *P* value less than 0.05 was considered statistically significant.

Results

Mild COVID-19 patients have higher levels of MCP-1

According to the basic information of severe COVID-19 and mild COVID-19 patients in Table 1, it was suggested that there were no remarkable differences between them from hospital time and some complications by statistically analysis. In order to further clarify the different role of chemokine in severe and mild COVID-19 patients, we detected three chemokine (including MCP-1, IP-10 and IL-8) in the serum

among either severe, mild COVID-19 patients, or healthy controls. It was demonstrated that MCP-1 upregulation was observed in almost all COVID-19 patients no matter what is severe (296.7pg/mL±128pg/mL) or mild cases (215.9pg/mL±67.4pg/mL) than that of healthy control (36.2pg/mL±6.7pg/mL) (Fig. 1A) (P<0.01). In particular, MCP-1 has also shown higher expression in patients with mild cases of COVID-19. IP-10 showed upregulation in severe COVID-19 patients (199.2pg/mL±82.6pg/mL) while no difference in mild patients (51.7pg/mL±39.4pg/mL) than that of healthy control (37.1pg/mL±3.1pg/mL) (Fig. 1B). Meanwhile, severe COVID-19 patients also showed the upregulation of IL-8 (51.3pg/mL±12.4pg/mL vs 27.2pg/mL±5.4pg/mL in healthy controls) (Fig. 1C). Recent reports indicated that MCP-1, IP-10, and IL-8 levels were higher in COVID-19 patients and even higher among those admitted to ICU[10]. We also found that the level of expression of the three chemokine was increased in severe COVID-19 patients. It has been suggested that chemokine may play an important role in patients with severe COVID-19. The upregulation of MCP-1 in mild COVID-19 suggested that it may play the role in pathogenesis of mild diseases.

CCR2 show greater expression in PBMC from mild COVID-19 patients

Chemokines, when combined with corresponding receptors, play a chemotactic role in immune cells. In order to further clarify the role of chemokine in mild COVID-19 diseases, we tested the expression level of the receptors for MCP-1, IP-10, and IL-8, respectively. The expression level of the receptor of MCP-1, C-C motif receptor 2 (CCR2); the receptor of IP-10, chemokine (C-X-C motif) receptor 3 (CXCR3); and the receptor of IL-8, CXCR2 were assessed in the PBMC from mild COVID-19 patients and healthy controls. We observed the upregulation of CCR2 (5.28±1.89 vs 1.9±0.57) (Fig. 2A) (P <0.01), while there was no difference of CXCR3 (2.89±0.7 vs 2.58±1.0) (Fig. 2B) and CXCR2 (3.71±0.91 vs 2.96±1.02) (Fig. 2C) between mild COVID-19 patients and healthy controls. It was demonstrated that MCP-1 may participate in the pathogenesis of mild COVID-19 diseases by binding its receptor.

Higher expression of MCP-1 in mild COVID-19 patients is correlated with the inhibition of IFN signaling

Transcriptional activation of interferon regulator factors (IRFs) results in the launch of general antiviral programs. We then explored the expression levels of IRF3 in mild COVID-19 patients. The expression of IRF3, an important gene in the interferon signaling pathway, was down-regulated (0.67±0.35 vs 1.12±0.21) (Fig. 3A) (P<0.01). Meanwhile, the downregulation of IFN-β was observed in mild COVID-19 patients (31.6±3.7 vs 47.3±6.9) (Fig. 3B) (P<0.01). To clarify the relationship between serum MCP-1 and expression level of IRF3, we performed Spearman rank correlation analysis using SPSS software, the results showed that IRF3 downregulation was significantly negative correlated with MCP-1 (Fig. 3C) (P<0.01, r^2=0.861). Our results suggest that MCP-1 may be an effective index for mild COVID-19 patients and higher expression of MCP-1 in mild COVID-19 patients is correlated with the inhibition of IFN signaling.

Discussion

Previous studies have shown that elevated levels of pro-inflammatory cytokines, such as IFN-γ, TNF-α, IL-6 and IL-8, are associated with severe lung injury and adverse outcomes of SARS-CoV or MERS-CoV infection[18-20]. It has also demonstrated that severe COVID-19 patients have higher concentrations of chemokine in the serum than mild cases, suggesting that the magnitude of cytokine storm is associated with the disease severity[6,7].

Most of COVID-19 patients in Shiyan city are mild cases[4]. In order to further study the level of chemokine in mild COVID-19 patients, we detected the level of chemokine in the serum of mild COVID-19 patients admitted to Xiyuan Hospital and Renmin Hospital in Shiyan City. We selected monocyte chemokine, MCP-1, interferon induced protein 10, IP-10 and neutrophil chemokine, IL-8. MCP-1, this protein belongs to the C-C chemokine family and is a powerful monocyte chemotactic factor that is constitutively produced or induced by oxidative stress, cytokines, or growth factors. Monocytes and macrophages are the main source of MCP-1, which regulates the migration and infiltration of monocytes, memory T cells, and NK cells[21]. Huang et al. found that MCP-1 levels were higher in patients with COVID-19 and even higher among those admitted to ICU[10]. It has been reported that MCP-1 increases rapidly in the early acute phase of infection and then progressively decreases with the advance of the disease[22]. Xiong et al. detected elevated levels of MCP-1 in the bronchoalveolar lavage fluid of patients with COVID- 19 and found it to be associated with the pathogeny of the virus[23]. Elevated levels of MCP-1 have also been detected in the lung tissue of patients infected with SARS-CoV-2[24]. IP-10 was initially identified as a chemokine whose secretion is induced by IFN-γ. IP-10 is secreted by neutrophils, endothelial cells, keratinocytes, fibroblasts, dendritic cells, astrocytes, and hepatocytes. Through its binding to chemokine receptor 3 (CXCR3), it regulates immune system responses by activating and recruiting leukocytes, including T cells, monocytes, and NK cells[25]. Therefore, IP-10 and CXCR3 play a key role in recruiting leukocytes to inflamed tissues and in perpetuating inflammation, thereby making a major contribution to tissue damage[25]. Serum IP-10 levels were found to be elevated in patients with COVID-19 and they have been found to be even higher in those who required ICU admission, suggesting a relationship with lung damage and disease severity[10]. Liu et al. associated elevated serum IP-10 levels with a higher viral load and greater lung damage in patients with SARS-CoV-2[26]. Recent reports suggested that the expression level of IL-8 was higher in patients with severe COVID-19[10]. Our results also proved that MCP-1, IP-10 and IL-8 were up-regulated in severe COVID-19 patients (Fig.1). It has been suggested that chemokines may play an important role in patients with severe COVID-19. Meanwhile, MCP-1 has shown higher expression in patients with mild cases of COVID-19 (Fig. 1A). IP-10 and IL-8 showed upregulation in severe COVID-19 patients while no upregulation in mild patients (Fig. 1B, 1C).

In order to further clarify the role of chemokine in mild COVID-19 diseases, we tested the receptors for MCP-1, IP-10, and IL-8[27], respectively. Between mild COVID-19 patients and healthy controls, CCR2 was up-regulated (Fig. 2A) ($p<0.01$), while CXCR3 (Fig. 2B) and CXCR2 (Fig. 2C) have no difference. It was demonstrated that MCP-1 may participate in the pathogenesis of mild COVID-19 diseases by binding its receptor.

Engagement of virus-specific RNA structures culminates in oligomerization of these receptors and activation of downstream transcription factors, most notably interferon regulator factors (IRFs) and nuclear factor κB (NF-κB). We found that, in mild COVID-19 patients with higher level of MCP-1, the expression of IRF3, an important gene in the interferon signaling pathway, was down-regulated (Fig. 3A). The expression level of NF-κB did not found between them (data not shown). IFN-β levels in serum of peripheral blood from COVID-19 patients were lower than that of healthy controls (Fig.3B). Meanwhile, IRF3 downregulation was significantly negative correlated with the level of MCP-1 (Fig. 3C). We also check the level of IFN-β in the severe COVID-19 patients, but there was remarkable downregulation in severe COVID and there was no relationship with the expression of IRF3 (data not shown). So we regarded that the higher expression of MCP-1 in mild COVID-19 patients may be correlation with the inhibition of IFN signaling. Michael J, et al[28] demonstrated that IFN-β can induce MCP-1 transcription in bone marrow derived macrophages (BMDMs). Our results demonstrated a negative correlation of the level of MCP-1 in peripheral blood with the level of IRF3 expressed in PBMCs from mild COVID-19 patients in Shiyan. We have not carried out a separate analysis of macrophages in PBMCs, and its specific mechanism needs further analysis.

Other studies have already established that SARS-CoV-2 has greater sensitivity to type I IFN than SARS-CoV. In these studies, pre-treatment with IFN-α or IFN-β drastically reduced viral titers. These findings suggest that type I IFN may be effective as a prophylactic agent or an early treatment option for SARS-CoV-2. However, delayed IFN administration was of no benefit over a placebo[29]. Channappavanar et al. showed that delayed type I IFN expression can be detrimental in mice in the context of SARS-CoV-1 infection[30]. The timing of interferon exposition may be critical for controlling the virus and avoiding immunopathogenesis. Early administration of IFN was slightly beneficial to reducing viral load and improving clinical outcome in COVID-19 patients.

Conclusions

In summary, our findings add to our understanding of the immune-pathologic mechanisms of SARS-CoV-2 infection, and provide potential therapeutic targets and strategies. MCP-1 may be an effective indicator in mild patients, and early use of interferon has a good antiviral therapeutic effect.

Abbreviations

SARS-CoV: severe acute respiratory syndrome coronavirus; MERS-CoV: Middle East respiratory syndrome coronavirus; WHO: World Health Organization; IRF3/7: interferon regulatory factor 3/7; PBMCs: Peripheral blood mononuclear cells; ELISA: Enzyme linked immunosorbent assay; MCP-1: monocyte chemotactic protein 1; IP-10, Interferon-inducible protein-10; CCR2: C-C motif receptor 2; CXCR: chemokine (C-X-C motif) receptor. BMDMs: bone marrow derived macrophages.

Declarations

Ethics approval and consent to participate: The study protocol received approval from the Clinical Ethics Committee of Hubei University of Medicine (2020-TH-017). All individuals gave their informed consent to participate.

Consent for publication: Not applicable.

Availability of data and materials: All data generated or analyzed during this study are included in this published article.

Competing interests: The authors declare that they have no competing interests.

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Author Contributions: Conceived and designed the experiments: XX YW. Performed the experiments: XX, YG, MZ. Analyzed the data: XX BD, YW. Contributed reagents/materials/analysis tools: YW, GL. Wrote the paper: XX, BD.

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Figures

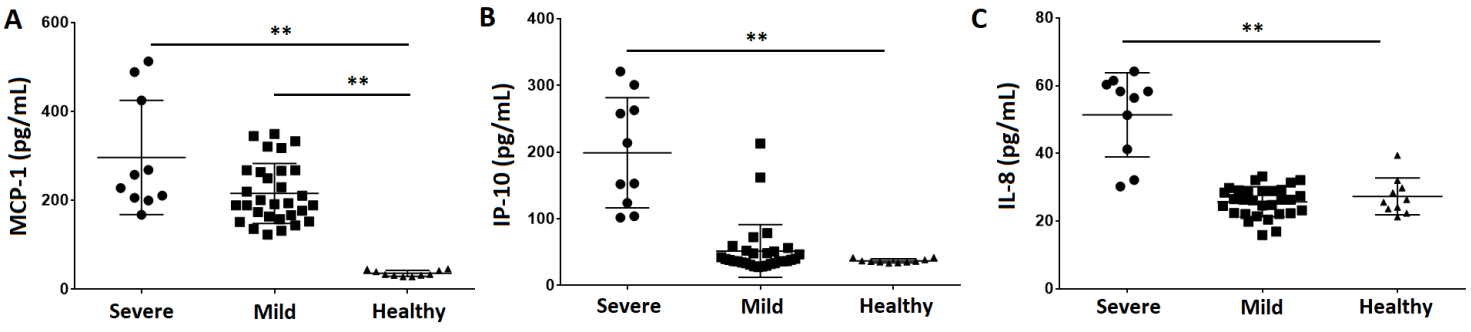

Figure 1

Mild COVID-19 patients have higher levels of MCP-1 (A). MCP-1 levels in serum of peripheral blood from COVID-19 patients (n=40) were higher than that of healthy controls (n=10). (B) IP-10 and (C) IL-8 levels in serum of peripheral blood from severe COVID-19 patients (n=10) were higher than that of the mild COVID-19 patients (n=30) and healthy controls (n=10). The chemokine levels in serum of study subjects were detected by ELISA according as the manufacturer's instruction. **denote that p<0.01

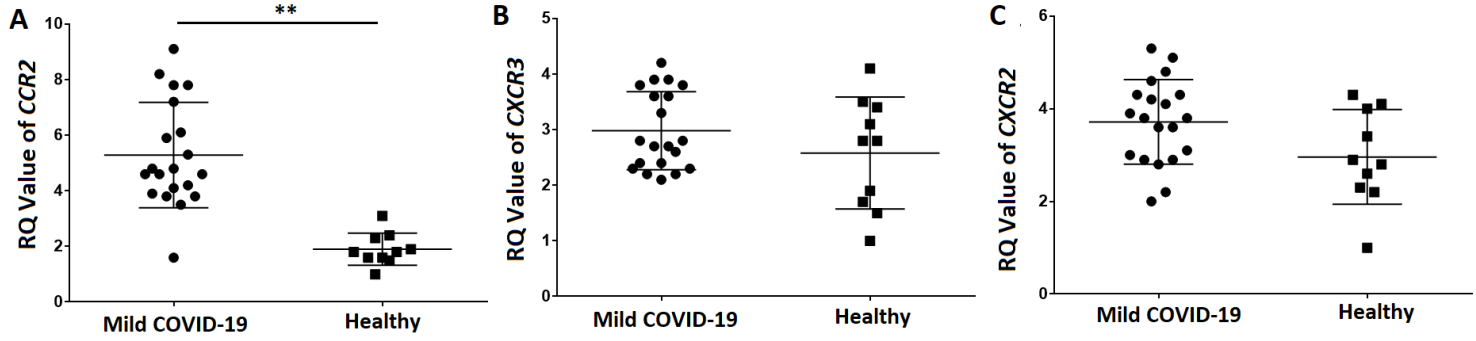

Figure 2

CCR2 show greater expression in PBMC from mild COVID-19 patients (A) The expression level of CCR2 is higher expressed in PBMC from mild COVID-19 patients. (B) CXCR2 and (C) CXCR3 have no difference between mild COVID-19 patients and healthy controls. After the RNA of PBMCs from mild COVID-19 patients (n=20) and healthy controls (n=10), it was reverse transcribed into cDNA. Real-time quantitative PCR was performed to quantify chemokine receptors and GAPDH levels. Each reaction tube contained 20μL of reaction mixture, including 10μL of SYBR Premix Ex Taq (TaKaRa, RR820A), 1μL of each 10μM primer, 1μL of cDNA template and 7μL of ddH2O. The program was performed as follows: denaturation at 95°C for 10 min, followed by 40 cycles of 15 s of denaturation at 95°C and 60 s annealing/extension at 56°C. All analysis used the Bio-rad CFX manager 3.1 and software GraphPad 5.0. **denote that p<0.01.

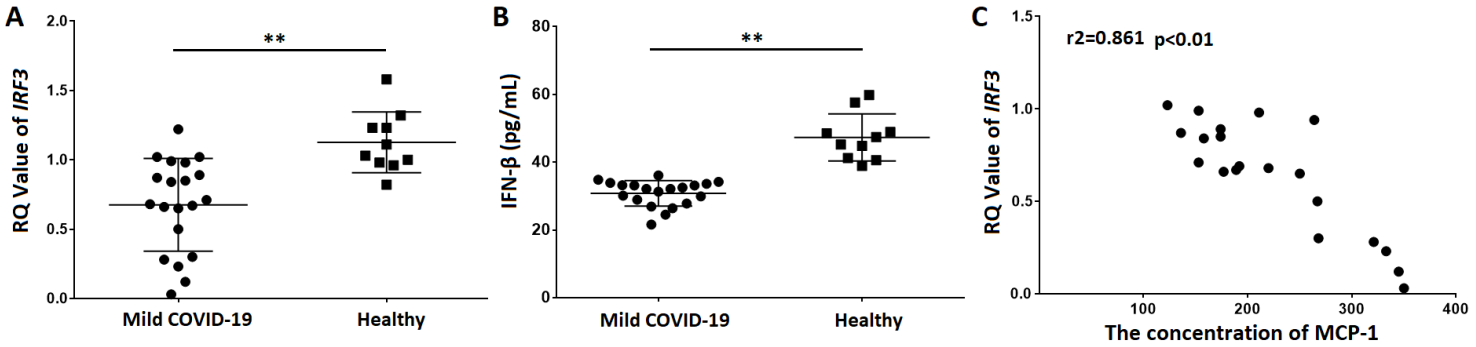

Figure 3

Higher expression of MCP-1 in mild COVID-19 patients is correlated with the inhibition of IFN signaling (A) IRF3 expression level has significant downregulation in mild COVID-19 patients. The RNA sample of PBMCs from mild COVID-19 patients (n=20) and healthy controls (n=10) was extracted and IRF3 were

detected by real-time PCR. (B) IFN-β levels in serum of peripheral blood from COVID-19 patients were lower than that of healthy controls. The cytokine levels in serum of study subjects were detected by ELISA according as the manufacturer's instruction. (C) Spearman rank correlation analysis in the SPSS software was performed to analyze the serum MCP-1 and the expression level of IRF3. It was showed that the expression IRF3 was significantly negative correlated with the concentration of MCP-1. The correlation coefficient r2 represents the degree of correlation. **denote that $p<0.01$. $r2=0.861$.