BMC Musculoskeletal Disorders

RESEARCH

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Significant correlations of upregulated MPO expression with cytokine imbalance in ankylosing spondylitis patients and the inhibitory effect mediated by mesenchymal stem cells

Shubei Liu[1*], Chunjuan Yang[2], Donghua Xu[2], Bingjie Gu[1*] and Minning Shen[1*]

Abstract

Background Little is known regarding both the role of myeloperoxidase (MPO) and the impact of mesenchymal stem cells (MSCs) on inflammatory and immune responses in ankylosing spondylitis (AS). This study is aimed to explore the role of MPO and the regulatory effect of umbilical cord-derived MSCs on MPO expression in monocytes in AS.

Methods MPO mRNA expression in the peripheral blood mononuclear cells (PBMCs) was detected by Real-time PCR. Cytokines including IL-2, IFN-γ, IL-17 A, IL-4, IL-10, IL-6 and TNF-α were determined by flow cytometry. A co-culture system was established by culturing THP-1 cells with MSCs at a ratio of 5:1.

Results Increased mRNA expression of MPO was observed in PBMCs of AS patients compared to healthy controls ($P < 0.05$). The mRNA expression of MPO was positively associated with C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) ($P < 0.05$) in AS. Besides, the levels of IL-2, IL-10, IFN-γ, IL-17 A, IL-4, IL-6, TNF-α in plasma were notably increased in AS ($P < 0.05$). Positive correlations between MPO expression and IL-2, IFN -γ, IL-4, TNF-α as well as IL-6 were demonstrated in AS ($P < 0.05$). Furthermore, MSCs remarkably suppressed the mRNA expression of MPO along with the secretion of IL-17 A and TNF-α, but promoted IL-10 generation in monocytes.

Conclusion MPO expression is significantly upregulated and correlates with cytokine imbalance in AS. It may serve as a valuable immunotherapeutic target for AS. MSCs can significantly inhibit monocyte-mediated inflammatory response potentially by downregulating MPO in monocytes.

Keywords Ankylosing spondylitis, Myeloperoxidase, Mesenchymal stem cells, Cytokines

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Introduction

Ankylosing spondylitis (AS) is a chronic and inflammatory disease with involvement of the spine, ligaments and tendons [1]. It belongs to axial spondylarthritis together with non-radiographic axial spondylarthritis [2]. Despite extensive research, the etiology and pathogenesis of AS remain not clearly defined. Several hypotheses have been proposed in the past few years, such as arthritogenesis, misfolding, molecular mimicry, and HLA-B27 homodimer [3]. High inflammation level and the imbalance of cytokine network are involved in the onset and progression of AS [1, 3]. The treatment methods of AS include exercise, physical therapy, medications, and surgery [2]. Pharmacotherapy is the main treatment for AS, of which tumor necrosis factor-α (TNF-α) inhibitors, interleukin-17 (IL-17) inhibitors and non-steroidal anti-inflammatory drugs (NSAIDs) are most widely used. However, all kinds of these drugs have side effects and have limited effects in part of AS patients [4–6]. Therefore, it is of great importance to identify more effective treatments for AS.

Myeloperoxidase (MPO) belongs to the heme peroxidase superfamily, which is encoded by the gene located on chromosome 17q [7, 8]. MPO is synthesized mostly by the azurophilic granules of neutrophils, while monocytes and macrophages can also produce MPO under the pathological conditions [9–11]. MPO has been previously found to play a pathogenic role in a variety of diseases, such as atherosclerotic disease and viral infections [7]. MPO has also been demonstrated to drive autoimmune inflammation in some autoimmune diseases characterized by chronic inflammation, such as multiple sclerosis (MS) and rheumatoid arthritis (RA) [12–14]. However, little is known of the role of MPO in AS.

Mesenchymal stem cells (MSCs) confer anti-inflammatory and immunoregulatory functions [15]. They originate from the mesoderm and can be found in various tissues, such as placenta, amniotic fluid, bone marrow, fatty tissue, and muscle [15, 16]. Under specific conditions, MSCs possess the capability of multi-lineage differentiation into cartilage, bone, and others, facilitating the repairment and regeneration of various tissues and organs [15]. Besides, MSCs can exert immunoregulatory effects through cell-to-cell contact or paracrine pathway in autoimmune disorders, such as systemic sclerosis (SSc), systemic erythematosus lupus (SLE), and RA [17–19]. There are also emerging findings which have supported MSCs as a promising therapy of AS [20–22]. However, the impact of MSCs on inflammation and immunological responses in AS remains largely unknown.

This study is aimed to clarify the regulatory effect of MPO in monocytes in AS, and its relationship with the immunological indexes of AS. In addition, the influence of MSCs in MPO-mediated biological effect in AS has also been investigated in order to provide new insight into optimizing the biological therapy of AS based on MSCs.

Methods

Subjects

As presented in Tables 1, 26 AS patients registered in Weifang People's Hospital, Shandong Second Medical University from April, 2022 to July, 2022 were included into this study as experimental group. 21 age- and sample size-matched healthy controls registered in the hospital for physical examination at the same time period were selected as controls. All AS patients were diagnosed according to the New York criteria revised by the American College of Rheumatology in 1984. The exclusion criteria were as follows: AS patients received biological therapy during the past one year; patients with RA; idiopathic inflammatory myopathy; SLE and other rheumatic diseases; patients with infectious diseases; pregnant or lactating females. Fresh blood samples were obtained from all participants followed the informed consent. The entire study was conducted under supervision of the Medical Ethics Committee of Weifang People's Hospital, Shandong Second Medical University. Information of gender, age, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), platelet count (PLT), and other terms were recorded (Table 1).

Peripheral blood mononuclear cells (PBMCs) isolation

Plasma samples were collected from the fresh peripheral blood samples of AS patients and controls by centrifugation at 3000 rpm for 10 min, and were stored at -80 °C. The remaining blood was adding with equivalent phosphate-buffered saline (PBS) solution, which was then gently shifted onto the upper layer of 5 ml Ficoll-Paque

Table 1 Characteristics of all participants ($\bar{x}$±SEM)

	AS ($n=26$)	Control ($n=21$)	Statistic parameter	*P* value
Sex (male/female)	19/7	9/12	$\chi^2=4.405$	0.036
Age (year)	43.769 ± 2.783	45.714 ± 2.673	$t=0.496$	0.622
CRP (mg/L)	12.206 ± 3.110	< 1	-	-
ESR (mm/H)	17.320 ± 3.652	< 0.5	-	-
PLT (10^9/L)	248.840 ± 10.004	236.571 ± 11.019	$t=0.825$	0.414
Disease duration (year)	7.337 ± 1.524	-	-	-

solution (Beyotime Biotechnology, Shanghai, China) in the centrifuge tube. The middle, white, and cell-containing layer was carefully aspirated following the centrifugation for 30 min at 2000 rpm. PBMCs were washed with PBS twice and used for the experiments.

Real-time quantitative polymerase chain reaction (RT-qPCR)

We use Trizol reagent (Invitrogen, CA, USA) to isolate total RNAs from PBMCs. To define the expression of MPO mRNA in PBMCs, RT-qPCR assay was performed by use of the reverse transcription RT kit (Vazyme, Nanjing China) and SYBR Green Mastermix kit (Vazyme Biotechnology, Nanjing, China) following the instructions. The relative expression of MPO mRNA was calculated by $2^{-\Delta\Delta Ct}$. Human gene primers were as follows. For MPO, upstream primer, TGCTGCCCTTTGACAACCT G, downstream primer, GCTCCCGAAGTAAGAGGGT. For GAPDH: upstream primer, GGAGCGAGATCCCTC CAAAAT, downstream primer, GGCTGTTGTCATACT TCTCATGG.

Cytokine assay

The concentrations of IL-2, IFN -γ, IL-17 A, IL-6, TNF-α, IL-4, and IL-10 in plasma were detected by the flow cytometric bead array (QuantoBio, Beijing, China) according to the product manual. Data were then analyzed using the FCAP Array (Version 3.0.19.2091) software.

Isolation and culture of human umbilical cord MSCs

The wharton's jelly was collected from the fresh umbilical cord by removing the membrane and blood vessels, which was cut into pieces and put into the culture flasks with DMEM-F12 medium (Gibco, USA) and 10% fetal bovine serum (Gibco, USA). The flasks were gently shaken to make the tissue blocks evenly dispersed at the bottom. Then, the cells were cultured in the incubator under 5% CO_2 at 37℃ to make the tissue blocks fully adhere to the wall. Change the DMEM-F12 complete medium every 3 days. When cells grow to 80%, they were subcultured for subsequent experiments. Usually, the 3–5 generation of cells were used for subsequent experiments in this study. As demonstrated previously, human umbilical cord MSCs were highly expressed with CD73, CD105, CD90, and CD44, but lowly expressed with CD45 and CD34 [23]. They also possess the capacity of differentiation into adipocytes, osteocytes and chondrocytes under certain conditions [23].

THP-1 cell culture

$2\text{-}5 \times 10^5$/ml THP-1 cells were cultured under 5% CO_2, 37℃. The culture medium in flasks was changed every 3 days when the cell fusion reached 80%. DMEM-F12 culture medium (Gibco, USA) containing 10% FBS (Gibco, USA) was used.

Construction of MSC and THP-1 cell co-culture system

MSCs in logarithmic phase of growth were harvested by centrifugation and resuspended in DMEM-F12 incomplete culture medium (Gibco, USA). 1.2×10^5 cells/well MSCs were planted into plates and cultured at 37 °C, 5% CO_2 overnight to make the cells adherent to the wall. Then, 6×10^5 cells/well THP-1 cells in logarithmic phase of growth were added into MSC cell-cultured wells to construct the 1:5 co-culture system. Four groups were set up with or without the stimulation of LPS (1 μg/mL). The four groups were as follows: THP-1 cells blank group, LPS-stimulated THP-1 cells group, THP-1 + MSC co-culture group, and LPS + THP-1 + MSC group. After 24 h of incubation, the cultural supernatant and THP-1 cells were collected for subsequent detections.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 8.0.2 software. Continuous data were described by $\bar{x}$ ±SEM. According to the status of normal distribution, Mann-Whitney U test or independent samples unpaired t-test was selected for different contrasts between groups. One-way ANOVA was used for comparison of means more than two groups. Pearson correlation analysis or spearman correlation analysis was applied for the correlation analyses according to the status of normal distribution. Differences were statistically significant at $P < 0.05$.

Results

MPO expression in PBMCs from AS patients and healthy controls

The RT-qPCR assay results revealed that the mRNA expression of MPO in PBMCs from AS patients was prominently elevated compared to the healthy controls ($P < 0.001$) (Fig. 1).

Correlation between MPO expression and the inflammatory biomarkers of AS

Pearson's correlation analysis showed a positive association of MPO mRNA expression with CRP and ESR in AS, respectively, while no significance was observed regarding age and platelet counts (Fig. 2A-D). It could be concluded that MPO was associated with the inflammatory status of AS.

Changes of cytokines in plasma samples from AS patients

The cytokines levels in plasma samples were analyzed by flow cytometry (Table 2). Compared to healthy controls, plasma levels of IL-2, TNF-α, IFN-γ, IL-4, IL-10, IL-6, and IL-17 A were obviously increased in AS patients (Fig. 3,

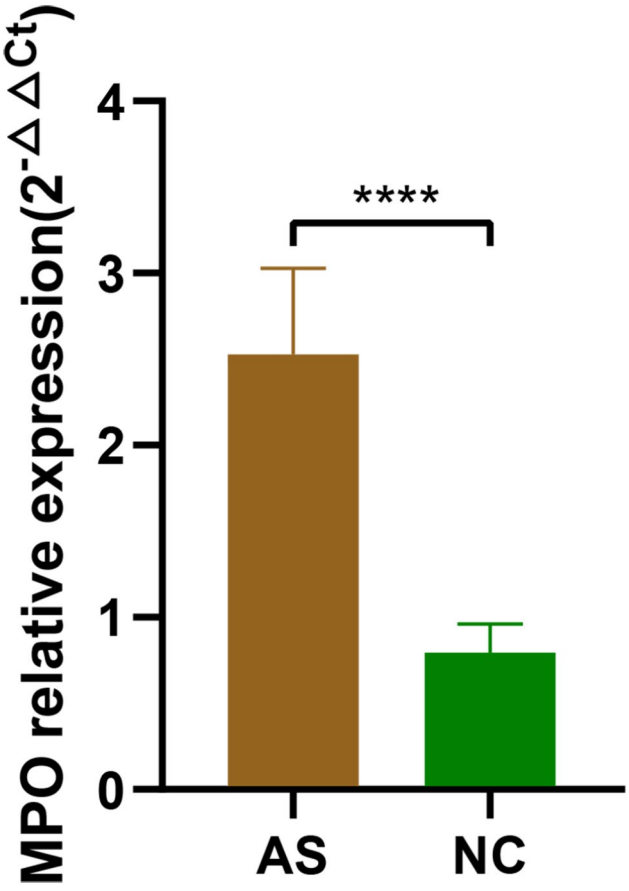

Fig. 1 MPO mRNA expression in PBMCs from experimental group and controls. (AS, ankylosing spondylitis; NC, normal controls; Mann-Whitney U test, ****, $P < 0.0001$)

all $P < 0.0001$) (Table 2; Fig. 3A-G), suggesting notable immune disorders and immune inflammation in AS. As M2 macrophage-related cytokines, IL-4 and IL-10 were increased in AS, which may be a compensatory increase to promote tissue regeneration and repair.

Association between MPO and Immunoinflammatory cytokine in AS

As shown in Fig. 4A-G, the MPO mRNA expression in PBMCs in AS was positively associated with the plasma IL-2 ($r = 0.5544$, $P = 0.0049$), IL-4 ($r = 0.4184$, $P = 0.0321$), IL-6 ($r = 0.7435$, $P < 0.0001$), TNF-α ($r = 0.5372$, $P = 0.0099$), and IFN -γ ($r = 0.4845$, $P = 0.0164$). However, no remarkable correlation between MPO and IL-10, IL-17 A was observed in AS, as shown in Fig. 4D and G. Taken together, these findings have suggested that MPO is involved in AS by regulating the balance of cytokines and cytokines-mediated immune inflammation, thereby influencing the onset and progression of AS.

Regulatory effect of umbilical cord-derived MSCs on MPO expression in monocytes

MSCs have anti-inflammatory, immunomodulatory, and regeneration effects, serving as one of the most promising therapeutic options for refractory autoimmune diseases, including AS. THP-1 is a human monocytic leukemia cell line, which can be differentiated into macrophage-like cell stimulated by a variety of stimulators. The model of LPS-stimulated THP-1 cell is generally accepted to study the regulatory effects of monocytes and macrophages [24]. In this study, we have found umbilical cord-derived MSCs exert significantly inhibitory effect on MPO mRNA expression in LPS-stimulated THP-1 cells (Fig. 5). The mRNA expression of MPO in THP-1 cells was significantly promoted by LPS, whereas MSCs significantly inhibited the mRNA expression of MPO in THP-1 cells induced by LPS after co-cultured these cells. Accordingly, MSCs can alleviate inflammation in AS by inhibiting MPO-mediated biological effects.

Altering effect of umbilical cord-derived MSCs on cytokine secretion in LPS-stimulated THP-1 cells

IL-2, TNF-α, IFN-γ, IL-4, IL-10, IL-6, and IL-17 A concentrations in the supernatant of co-culture system were shown in Table 3; Fig. 6A-G. As a Th2 cytokine, IL-4 showed a negative feedback effect in the interplay between MSCs and THP-1 cells (Fig. 6B). Substantial production of IL-6 was observed in THP-1 cells after MSC intervention, suggesting the critical role of IL-6 in maintaining the biological function of MSCs (Fig. 6C). LPS significantly induced higher secretion of TNF-α and IL-17 A, two pro-inflammatory cytokines, but lower anti-inflammatory cytokine IL-10 in the cultural medium (Fig. 6D, E, G). The roles of anti-inflammatory cytokines IL-4 and IL-10 in AS pathogenesis have not been well revealed. In this study, we found increased levels of IL-4 and IL-10 in the peripheral blood samples from AS patients, suggesting a negative feedback regulation in the body for self-protection. However, when THP-1 cells were cocultured with MSCs, MSCs significantly inhibited the secretion of IL-17 A and TNF-α in LPS-stimulated THP-1 cells, but promoted the production of IL-10. There was significantly increased level of IL-6 but no other cytokines in the cultural medium of MSCs compared the NC group, suggesting the underlying role of IL-6 during MSC growth and function. However, no significant difference of IL-6 and other cytokines between groups of MSC and MSC/LPS, which implicated that the cytokine release of THP-1 cells was not significantly influenced by MSC. MSCs could notably inhibited the production of proinflammatory cytokines in THP-1 cells under the stimulation of LPS. As a result, MSCs exert a significantly anti-inflammatory effect in AS by

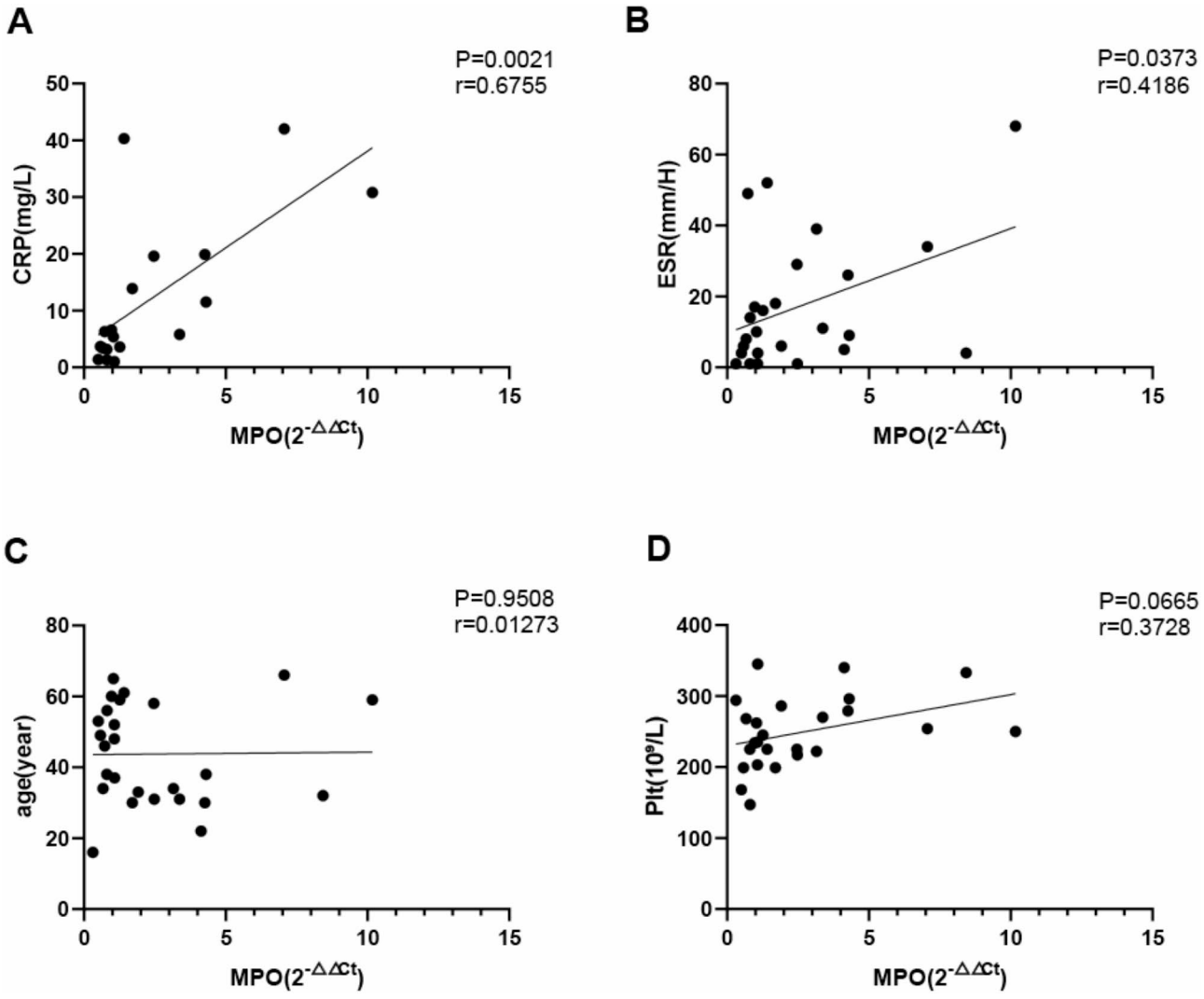

Fig. 2 Correlation of MPO mRNA expression level with AS clinical indexes. (**A**) The MPO mRNA level in AS was positively associated with the consistence of CRP ($n=18$, $P=0.0021$); (**B**) The MPO mRNA level in AS was positively linked with consistence of ESR ($n=25$, $P=0.0373$); (**C**) The MPO mRNA level in AS had no significant correlation with age ($n=26$, $P=0.9508$); (**D**) The mRNA level of MPO in AS was not statistically associated with peripheral blood platelet count ($n=25$, $P=0.0665$)

Table 2 Concentrations of plasma cytokines in AS patients and controls. ($\bar{x}$±SEM, pg/mL)

	AS ($n=25$)	NC ($n=10$)	*P* value
IL-2	5.302 ± 0.304	2.445 ± 0.156	< 0.0001
IL-4	3.261 ± 0.173	0.774 ± 0.130	< 0.0001
IL-6	4.025 ± 0.418	1.380 ± 0.157	< 0.0001
IL-10	3.253 ± 0.229	0.944 ± 0.245	< 0.0001
TNF-α	4.709 ± 0.568	1.255 ± 0.255	< 0.0001
IFN-γ	0.997 ± 0.065	0.306 ± 0.098	< 0.0001
IL-17 A	8.001 ± 0.650	1.198 ± 0.424	< 0.0001

downregulating MPO and proinflammatory cytokines in monocytes.

Discussion

Though the essential roles of immune dysregulation and inflammatory responses in AS have long been proposed, the pathogenesis of AS remains poorly understood. Elucidating the underlying mechanism regulating immunity and inflammation in AS is essential for the identification of more effective therapies for this disease. The findings from this study suggested that MPO expression was significantly upregulated and correlated with the cytokine imbalance in AS. These findings indicate that MPO may serve as a potential valuable immunotherapeutic target for AS.

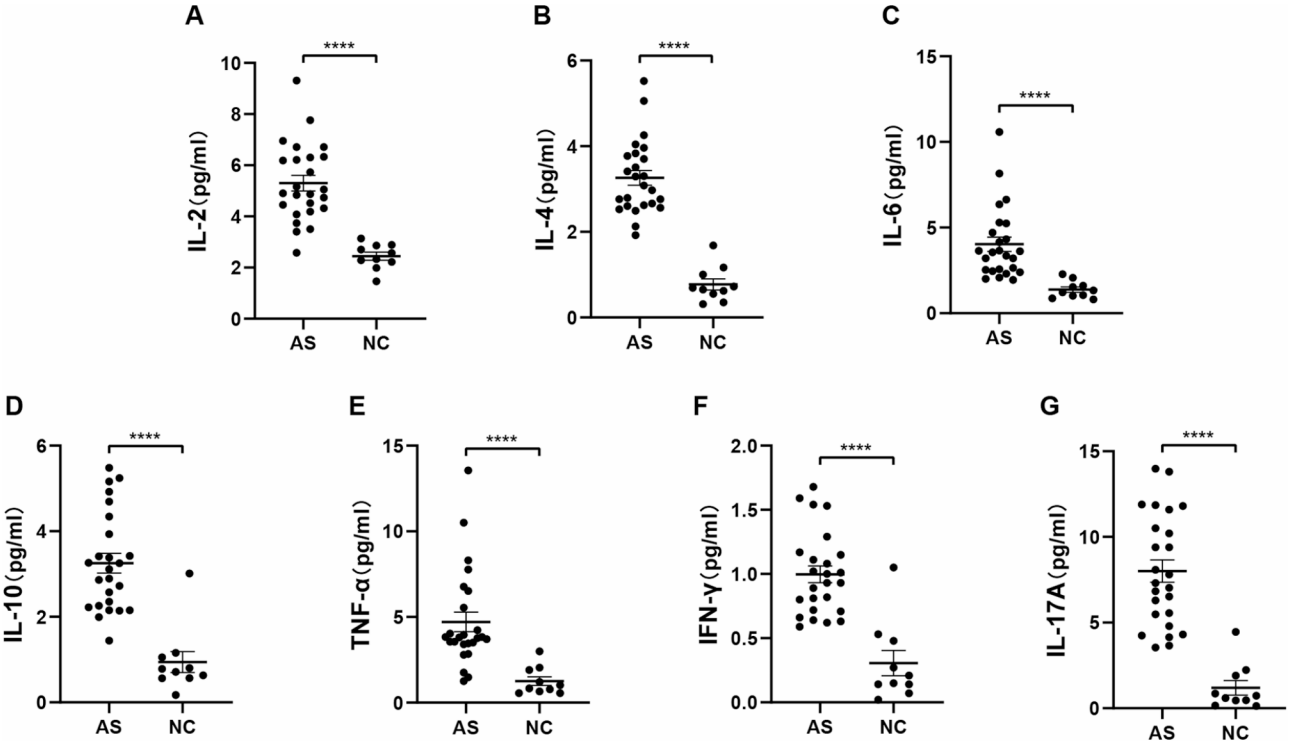

Fig. 3 Plasma cytokines difference between AS patients and controls. (**A**) The concentration of plasma IL-2; (**B**) The level of plasma IL-4; (**C**) The level of plasma IL-6; (**D**) The level of plasma IL-10; (**E**) The level of plasma TNF-α; (**F**) The level of plasma IFN-γ; (**G**) The level of plasma IL-17 A. (All comparisions were analyzed by Mann-Whitney U test, ****, $P < 0.0001$)

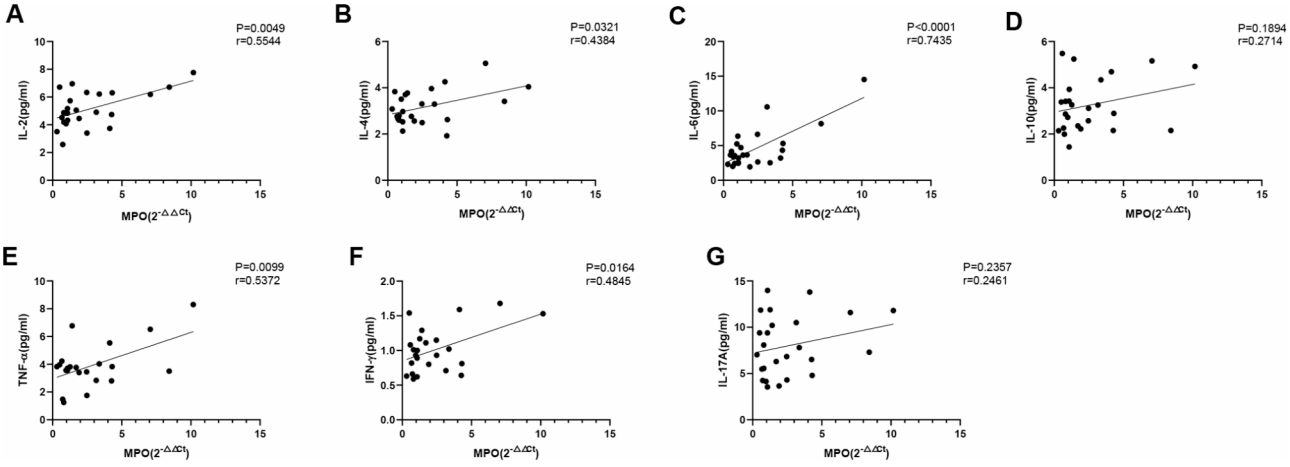

Fig. 4 Correlation analysis between MPO mRNA expression levels and plasma cytokine levels in AS patients. (**A**) MPO mRNA expression level in PBMCs was positively correlated with plasma IL-2 of AS patients, $n = 24$, $P = 0.0049$; (**B**) MPO mRNA expression level in PBMCs was positively correlated with plasma IL-4 of AS patients, $n = 24$, $P = 0.0321$; (**C**) MPO mRNA expression level in PBMCs was positively correlated with plasma IL-6 of AS patients, $n = 25$, $P < 0.0001$;(**D**) MPO mRNA expression level in PBMCs had no significant correlation with plasma IL-10 of AS patients, $n = 25$, $P = 0.1894$; (**E**) MPO mRNA expression level in PBMCs was positively correlated with plasma TNF-α of AS patients, $n = 22$, $P = 0.0099$; (**F**) MPO mRNA expression level in PBMCs was positively correlated with plasma IFN -γ of AS patients, $n = 24$, $P = 0.0164$; (**G**) MPO mRNA expression level in PBMCs had no significant correlation with plasma IL-17 A of AS patients, $n = 25$, $P = 0.2357$

As a heme peroxidase, MPO is primarily expressed by neutrophils, monocytes, and macrophages [7]. Monocyte precursor cells are capable of synthesizing MPO during bone marrow development and maturation, while the synthesis usually terminates after the maturation of monocytes [25]. Nonetheless, under inflammatory or immune-activated conditions, monocytes and macrophages can both produce increased MPO, leading to inflammation and damages to normal tissues [7, 26]. In addition to producing excessive oxidants causing tissue

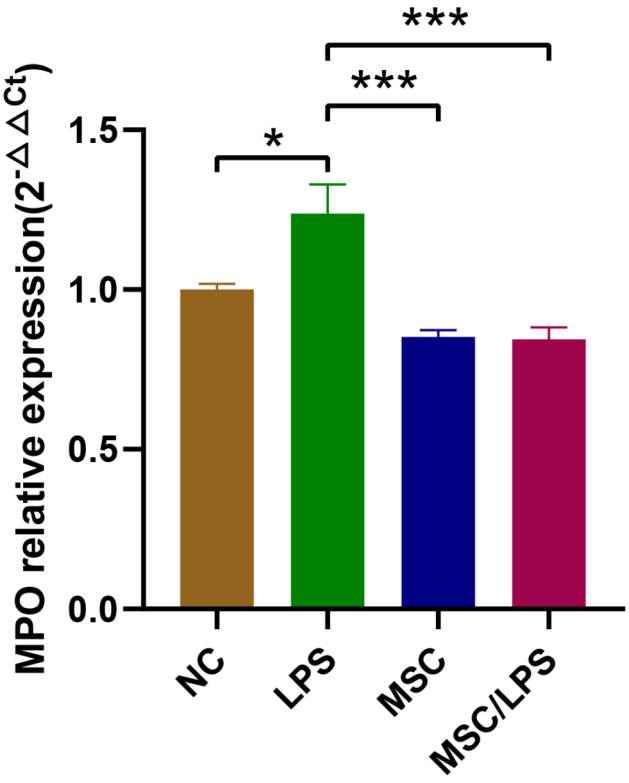

Fig. 5 MSCs inhibit the MPO mRNA expression in THP-1 cells stimulated by LPS. (*, $P<0.05$; ***, $P<0.001$; $n=4$)

damages, MPO is also involved in immune and inflammatory responses through complicated signaling cascades [27, 28]. Therefore, MPO can exert a pathogenic effect in various diseases by regulating inflammation and immune homeostasis. However, there is no evidence supporting the role of MPO in the regulation of monocyte- and macrophage-mediated immune effects in AS.

The expression of MPO in AS patients have been explored by few studies. The study by Yu et al. has implicated MPO as a potential biomarker for AS, which is upregulated in the hip ligaments of AS patients and participates in the regulation of the phagosome pathway activation [29]. Moreover, the higher expression of MPO has been demonstrated to significantly promote fibroblast activation and inflammatory response in AS patients with hip joint involvement [29]. Similarly, our study revealed a significantly increased expression of MPO at the mRNA level in PBMCs from AS patients compared to the healthy controls, and a positive relationship of MPO with inflammation- and disease activity-associated indexes in AS including CRP and ESR ($P<0.05$). Both CRP and ESR are utilized as markers in calculating the Ankylosing Spondylitis Disease Activity Score (ASDAS) [30]. However, some patients may present with symptoms, such as pain and morning stiffness, even though they have normal ESR and CRP in serum, highlighting the necessity of identifying new biomarkers for the disease activity estimation [31]. The present study has supported a critical role of MPO as a biomarker for autoimmune inflammation in AS, and it may serve as a valuable indicator for the inflammatory status of AS.

A great deal of evidence has suggested that various inflammatory cytokines are involved in the development and progression of AS [1, 3]. Among those cytokines, TNF-α and IL-17 A are key and successful targets for biological targeted therapy, and monoclonal antibodies or antagonists targeting TNF-α and IL-17 A have been commonly used as effective remedies for AS patients [2]. TNF-α, mainly produced by activated monocytes and macrophages, can promote osteoclast differentiation, and affect bone formation by inhibiting alkaline phosphatase activity and the expression of osteocalcin and Runx2 in osteoprogenitor cells, serving as a key immunotherapeutic target for AS [1, 3]. Currently, there are a number of TNF-α inhibitors widely used for the alleviation of symptoms and the restoration of cytokine balance among AS patients [32–34]. For IL-17, disruption of the IL-17/IL-23 pathway can lead to various autoimmune diseases including AS and psoriasis, highlighting the significant role of IL-17 A in AS pathogenesis [3, 35]. Apart from TNF-α and IL-17 A, other inflammatory cytokines are also possibly involved in AS pathogenesis, such as IL-6 and IFN-γ [1, 3]. However, the therapy by targeting IL-6 or IFN-γ is not effective in AS patients. Thus, more effective targets for biological targeted therapy need to be identified for AS. It has been found that M2 macrophages are prevalent in AS lesions by producing anti-inflammatory cytokines of IL-4 and IL-10, which contributes to the tissue

Table 3 The concentrations of IL-2, TNF-α, IFN-γ, IL-4, IL-10, IL-6, and IL-17 A in the supernatants of 4 groups. ($\bar{x}$±SEM, pg/mL)

	NC	LPS	MSC	MSC/LPS	*P* value
IL-2	1.095 ± 0.034	1.273 ± 0.105	1.093 ± 0.070	1.018 ± 0.152	0.423
IL-4	0.357 ± 0.103	0.668 ± 0.050	0.380 ± 0.048	0.290 ± 0.084	0.010
IL-6	5.412 ± 3.304	7.193 ± 3.257	146.352 ± 50.264	159.534 ± 53.957	0.004
IL-10	1.435 ± 0.065	0.395 ± 0.225	1.434 ± 0.123	1.460 ± 0.188	0.016
TNF-α	1.257 ± 0.093	2.300 ± 0.260	1.317 ± 0.176	1.140 ± 0.070	0.027
IFN-γ	0.190 ± 0.060	0.240 ± 0.034	0.170 ± 0.063	0.190 ± 0.063	0.853
IL-17 A	0.220 ± 0.095	1.290 ± 0.059	0.325 ± 0.087	0.230 ± 0.134	< 0.0001

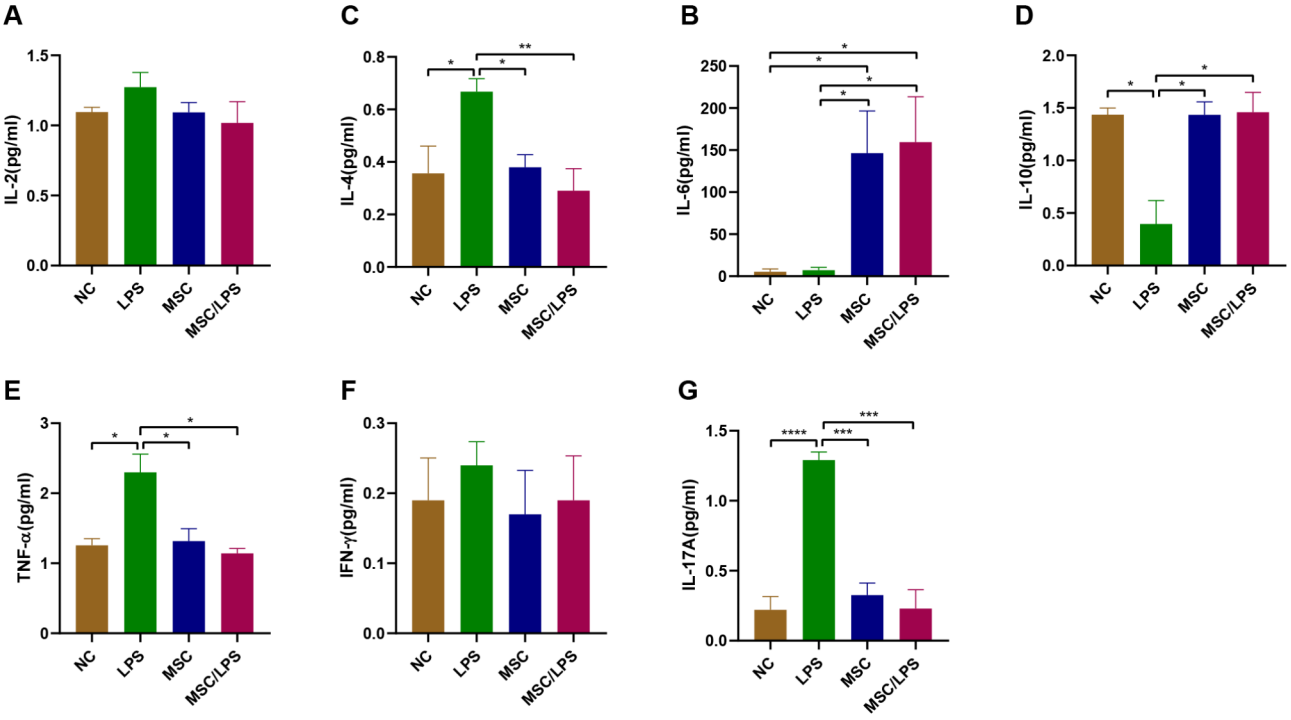

Fig. 6 Altering effects of umbilical cord-derived MSCs on cytokines production of THP-1 cells stimulated by LPS. (**A**) The concentration of IL-2 in the supernatant of the co-cultured system: t-test, $P > 0.05$, $n = 3$; (**B**) The level of IL-4 in the supernatant of the co-cultured system; (**C**) The level of IL-6 in the supernatant of the co-cultured system; (**D**) The level of IL-10 in the supernatant of the co-cultured system; (**E**) The level of TNF-α in the supernatant of the co-cultured system; (**F**) The level of IFN-γ in the supernatant of the co-cultured system; (**G**) The level of IL-17 A in the supernatant of the co-cultured system (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; $n = 3$)

regeneration and repair in AS [1, 3]. In our study, the pro-inflammatory cytokines of IL-6, TNF-α, IFN-γ, and IL-17 A were prominently elevated in the plasma samples from AS patients ($P < 0.05$), while the level of anti-inflammatory cytokine IL-10 was also increased ($P < 0.05$), suggesting a notable immune imbalance in the peripheral circulation of AS patients. IL-10 might play a negative feedback regulation. All these findings have suggested the potential role of MPO in regulating the cytokine balance and immune response in AS. Accordingly, MPO may play a role in pathogenesis of AS and serve as a valuable target for the treatment of AS. How MPO regulates inflammation and immunity in AS warrants to be explored in more future studies.

Accumulated studies have shown that MSCs confer anti-inflammatory, anti-infectious, and immunomodulatory effects by inhibiting T and B cell responses and the proinflammatory cytokines production, and maintaining the homeostasis [36–38]. The study by Shen et al. has reported that MSCs induced the Th2 cells polarization in the spleens of AS model mice but exert no effects on the differentiation of Th17 and Treg cells [20]. Clinical case reports have documented significant alleviation of AS symptoms following stem cell transplantation for multiple myeloma [39]. Our findings have firstly suggested that human umbilical cord-derived MSCs not only significantly suppress the expression of MPO expression and the secretion of IL-17 A and TNF-α in monocytes stimulated by LPS, but markedly facilitate the production of IL-10. Therefore, MSCs may exert a therapeutic effect on AS by inhibiting MPO expression in monocytes.

There are several limitations in this study. Firstly, we do not collect more baseline information of patients, such as the disease activity index BADAI. The potential mixed factors may affect the conclusions. The dysregulated expression of MPO is only confirmed in PBMCs of AS patients, without further illustration of its expression in specific immune cells in AS. There is also lacking further evaluation of MPO expression at the protein level. Secondly, we do not clarify the molecular mechanism by which MPO modulates immune and inflammatory responses of monocytes. The downstream signaling pathways and key targets of MSCs in regulating MPO need to be explored in further search. Thirdly, in the THP-1/MSC co-culture system, the dynamic changes of cytokine production at different time are not estimated. Therefore, the impact of feedback regulations between proinflammatory and anti-inflammatory cytokines during the interplay of monocytes and MSCs warrants further investigation. In future studies, we will further explore the effects and mechanisms of MSCs in regulating MPO to provide

updated insight into the optimized biological therapy of AS.

Conclusions

MPO is significantly upregulated in AS, which may affect the cytokine imbalance in AS. MPO serves as a potential valuable immunotherapeutic target for AS. Most importantly, MSCs can significantly inhibit monocyte-mediated inflammatory response and downregulate the expression of MPO, thereby may possess the potentials in alleviating AS symptoms and inhibiting disease progression. Nonetheless, the findings in this study warrant to be validated in deep research in vitro and in vivo.

Abbreviations

MPO	Myeloperoxidase
MSCs	Mesenchymal stem cells
AS	Ankylosing spondylitis
PBMCs	Peripheral blood mononuclear cells
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
TNF-α	Tumor necrosis factor-α
NSAIDs	Non-steroidal anti-inflammatory drugs
IL-17	Interleukin-17
MS	Multiple sclerosis
RA	Rheumatoid arthritis
SSc	Systemic sclerosis
SLE	Systemic erythematosus lupus
PLT	Platelet count
PBS	Phosphate-buffered saline
RT-qPCR	Real-time quantitative polymerase chain reaction
ASDAS	Ankylosing Spondylitis Disease Activity Score

Acknowledgements

Not applicable.

Author contributions

SL: study design, data collection, sample collection, experiments, statistical analysis and writing of the manuscript. CY: data collection and sample collection and experiments. DX: study design, experiments, statistical analysis and writing of the manuscript. GB, study design, supervision and writing of the manuscript. MS, study design, supervision and writing of the manuscript. All authors approved the final manuscript.

Funding

This work is supported by funds from the National Natural Science Foundation of China (No. 82171790).

Data availability

Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Medical Ethics Committee of Weifang People's Hospital, Shandong Second Medical University (date: 12.12.2021, No. 2021YX074). All patients provided informed consent, and the study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Clinical trial number

Not applicable.

Received: 4 September 2024 / Accepted: 19 February 2025
Published online: 01 March 2025

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