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REVIEW ARTICLE

TNFAIP3 regulates inflammatory arthritis through the differentiation of monocytes into macrophages

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Abstract

Background: Rheumatoid arthritis (RA) is a disease characterized by synovitis. The synovium of RA patients is rich in macrophages, which are differentiated mainly from monocytes. The susceptibility gene of RA, *tumor necrosis factor-α inducible protein 3* (*tnfaip3*), is considered an anti-inflammatory factor. Our previous study revealed the abnormal protein expression of TNFAIP3 in monocytes from patients with RA.

Objective: In the present study, we aimed to explore the role of TNFAIP3 in monocytes in RA and its potential functions.

Methods: *In vivo*, we injected adenoviral vectors overexpressing *tnfaip3* into mice with collagen-induced arthritis (CIA) (the TNFAIP3-oe group). Arthritis scores, as well as the expression of iNOS and CD206 in the synovium, were compared between the TNFAIP3-oe group and the CIA group. *In vitro*, we used lentivirus transfection to upregulate/downregulate the expression of *tnfaip3* in THP-1 cells. The ability of these cells to migrate, secrete cytokines and differentiate into macrophages was compared.

Results: Compared with that in the CIA group, arthritis in the TNFAIP3-oe group was ameliorated ($p = 0.030$). Moreover, the joints of these mice presented more $CD206^+$ cells and fewer $iNOS^+$ cells (both $p < 0.001$), indicating the anti-inflammatory effect of TNFAIP3 and its regulation of macrophage polarization. *In vitro*, the *tnfaip3*-depleted cells (the TNFAIP3-i group) had greater migration and differentiated into M1 macrophages, and more cells overexpressing *tnfaip3* (the TNFAIP3-oe group) differentiated into M2 macrophages. Furthermore, cells in the TNFAIP3-i group showed increased secretion of the proinflammatory cytokines IL-6 and MMPs.

Conclusions: Taken together, these findings suggest that TNFAIP3 in monocytes can regulate inflammatory arthritis by modulating monocyte migration, differentiation, and cytokine secretion.

Key words: Rheumatoid arthritis, TNFAIP3, monocyte, macrophage, inflammation

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Introduction

As a common inflammatory disease, rheumatoid arthritis (RA) is characterized by synovitis. Synovial hyperplasia and inflammatory cell infiltration are found in the joints of patients with RA. The hyperplastic synovium is composed mainly of synovial macrophages and fibroblast-like synoviocytes (FLSs). Most tumour necrosis factor (TNF)-producing cells are found in the synovium of RA patients via immunohistochemistry (IHC).[1] In contrast, macrophages, not fibroblasts, secrete increased TNF-α, which can induce the secretion of inflammatory cytokines, T-cell activation, and Toll-like receptor activation in the synovium.[2] Furthermore, macrophages can produce chemokines such as CX3CL1 and CCL2, which can increase the chemotaxis of monocytes to the synovium.[3] Moreover, macrophages can differentiate into osteoclasts.[4]

As the primary source of macrophages in the synovium, monocytes play an essential role in the pathogenesis of RA. On the one hand, monocytes express chemokine receptors and secrete proinflammatory cytokines.[5] On the other hand, monocytes can differentiate into macrophages and osteoclasts. In humans, there are three subtypes of monocytes: classical monocytes (CMs: $CD14^{++}CD16^{-}$), intermediate monocytes (IMs: $CD14^{+}CD16^{+}$), and nonclassical monocytes (NCMs: $CD14^{+}CD16^{++}$).[6] Our previous study revealed increased levels of monocytes, especially IMs and NCMs, in the peripheral blood of RA patients. As the most prominent monocytes in circulation, CMs are believed to differentiate into IMs and NCMs in the inflammatory environment. Previous studies have shown that IMs in the RA synovium secrete proinflammatory cytokines, can differentiate into macrophages and promote Th17 activation and expansion.[7] In addition, NCMs are considered responsible for an early inflammatory response.[8] Taken together, these findings indicate that monocytes play a proinflammatory role in RA.

Some susceptibility genes, such as *tumor necrosis factor-α inducible protein 3* (*tnfaip3*), have been found in RA through genome-wide association studies (GWASs).[9] The protein TNFAIP3, also called zinc finger protein A20, has seven Cys2-Cys2 zinc finger motifs in its C-terminus and a domain of cysteine protease deubiquitylating enzymes (DUBs) in its N-terminus. In addition, A20 has been proven to inhibit NF-κB activation through its regulation of ubiquitination.[10]

A previous study showed that myeloid-cell-specific deletion of *tnfaip3* in mice triggers spontaneous erosive polyarthritis that resembles that in humans.[11] Myeloid cells partly differentiate into monocytes and macrophages. However, studies of TNFAIP3 have focused on the dysfunction of macrophages but not monocytes in RA. However, monocytes play crucial roles in RA not only by secreting proinflammatory cytokines and migrating into the synovium but also by differentiating into M1 macrophages. Furthermore, as our previous study revealed abnormal expression of TNFAIP3 in the monocytes of RA patients, we hypothesized that monocyte functions are regulated by abnormal TNFAIP3 expression. Therefore, we aimed to clarify how abnormal expression of TNFAIP3 in monocytes contributes to synovitis in the present study.

Methods

Animal model

Fifteen DBA/1J mice were purchased from Cavins Laboratory Animal Co., Ltd. Collagen-induced arthritis (CIA) was induced out in the present study. Briefly, collagen type II (CII) (Chondrex, USA) was emulsified with Freund's complete adjuvant (Sigma–Aldrich, USA), and the prepared emulsion was administered to 10 male mice (6–8 weeks old) by intracutaneous injection. After 21 days, the mice received a second injection of CII emulsified with Freund's incomplete adjuvant (Sigma–Aldrich, USA). The mice were monitored for signs of arthritis onset on the basis of their clinical scores. Clinical arthritis was scored on a scale of 0 to 3, where 0 = no swelling, 1 = slight swelling and erythema, 2 = pronounced oedema, and 3 = joint rigidity. In addition, each limb was graded, and the grades were summed to yield the arthritis score for each animal. After 28 days, 2.5×10^{12} v.g/mL transfected adenovirus overexpressing *tnfaip3* (n = 5) or control adenovirus (n = 5) (Shanghai GeneChem Co., Ltd., China) was administered by tail vein injection. The mice were sacrificed after 60 days, and the limbs were collected.

Histological analysis

Formalin-fixed limbs were decalcified and paraffin-embedded using standard histologic techniques. For analysis of morphologic features, serial sections (4 μm) were prepared and stained with haematoxylin and eosin (H&E).

Formalin-fixed, paraffin-embedded tissue sections (3 μm) were placed on adhesive-coated slides. Next, the tissue sections were subjected to an antigen retrieval process. Briefly, the sections were immersed in EDTA buffer (pH 8.0) and heated for 2 min in a steamer. Next, the slides were incubated with monoclonal antibodies against iNOS (Abcam, England) and CD206 (Cell Signaling Technology, USA) in bovine serum albumin overnight at 4°C, followed by incubation with an Envision Detection System at room temperature for 20 min. Colour development was subsequently conducted by reacting with DAB solution for 10 min, followed by counterstaining with Harris haematoxylin. Finally, the sections were dehydrated, coverslipped, and observed under a light microscope. ImageJ was used to analyse the images.

Culture and transfection of THP-1 cells

THP-1 cells were purchased from the Chinese Collection of Authenticated Cell Culture. The cells were maintained in RPMI-1640 medium supplemented with 10% foetal bovine serum (FBS) (Gibco, Australia). The culture medium was changed every three days.

Lentiviruses were purchased from Shanghai Genechem Co., Ltd. Con-238 lentivirus was the control for the TNFAIP3-oe lentivirus, and con-077 lentivirus was the control for the TNFAIP3-i lentivirus. THP-1 cells were seeded into six-well plates (1×10^6 cells/well), and lentivirus was added to each well (1×10^6 cells/well). After 24 h, 500 μL of new culture medium was added to each well. After 5 days, the fluorescence of the cells was detected through a fluorescence microscopy, and the expression of TNFAIP3 was detected by flow cytometry.

Cell migration assay

Briefly, 0.5 mL of RPMI-1640 medium containing 10% joint fluid from RA patients was added to each well of a 24-well plate. A total of 1×10^4 cells were added to the transwells. After 24 h, the transwells were removed. The cells in the plates were observed. Furthermore, CCK-8 reagents (Beyotime Biotechnology, China) were added to the plates, and the OD values were recorded 1 h later.

RT-PCR

Briefly, 1 × 10^6 cells from each group were harvested and suspended in 500 μL of TRIzol (TaKaRa, Japan). Chloroform was added, and the mixture was vigorously mixed. The sample and TRIzol/chloroform mixture were subsequently centrifuged at 11,440 r/min for 15 min, the upper layer was collected, and an equal volume of isopropanol was added before incubation at room temperature for 10 min. Next, the samples were centrifuged at 11,440 r/min for 15 min, and the supernatant was discarded. The total RNA pellets were washed with 75% ethanol and dissolved in water supplemented with 0.05 g/L diethylpyrocarbonate (DEPC). RT-PCR was carried out in a 20-μL reaction system consisting of 10 μL of 2×One Step SYBR RT-PCR Buffer, 0.8 μL of PrimeScript 1 Step Enzyme Mix, 0.4 μL of ROX Reference Dye II, 2 μL of RNA, 1.6 μL of primer and 5.2 μL of RNase-free dH_2O (TaKaRa, Japan). All reactions were conducted in duplicate. The relative expression of the target genes was calculated using the $2^{-\Delta\Delta Ct}$ method, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene.

Flow cytometry

Briefly, 1 × 10^6 cells from each group were harvested and resuspended in 1 mL of PBS. The cells were labelled with antibodies against CD14 and CD16 (BD, USA) and incubated at 37°C in a humidified atmosphere containing 5% CO_2 for 30 min. Subsequently, the cells were washed with PBS, and CD14 and CD16 expression was detected.

Macrophage differentiation

THP-1 cells were seeded into 6-well plates and exposed to 100 ng/mL PMA (Sigma-Aldrich, USA) for 5 days. The culture medium was subsequently discarded, and adherent cells were washed with PBS and observed under a microscope. Then, the adherent cells were digested with 0.05% EDTA-trypsin (Gibco, Australia) for 3 min and washed with PBS three times. Finally, 500 μL of TRIzol was added to each well.

ELISA

The cell culture medium in the 6-well plates was collected. The levels of IL-1β, IL-6, and TNF-α in the supernatant were determined by ELISAs (R&D Systems, USA) according to the manufacturer's instructions.

Western blotting analysis

The cells were washed with ice-cold PBS and lysed in RIPA lysis buffer (Beyotime Biotechnology, China) for 30 min on ice. Equal amounts of proteins were subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels and then transferred onto nitrocellulose membranes. The blots were incubated with antibodies against p65 (Cell Signaling Technology, USA, Cat no. 8242), p-p65 (Cell Signaling Technology, USA, Cat no. 3033), and PPARγ (Abcam, UK, Cat no. ab178860). The blots were subsequently incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Abcam, UK; Cat no. ab97200). The immunoreactive bands were visualized using an enhanced chemiluminescence detection kit (Millipore, USA).

Statistical analysis

The data are expressed as the means ± standard errors of the means (SEMs). In the *in vivo* study, paired *t* tests were used to compare arthritis scores between the TNFAIP3-oe group and the CIA group. Comparisons of $iNOS^+$ and $CD206^+$ cells were performed via one-way ANOVA. In the *in vitro* study, all comparisons between two groups were made via unpaired *t* tests. All the statistical analyses were conducted via GraphPad Prism 8 software (GraphPad, USA). A p value < 0.05 was considered statistically significant.

Compliance with ethical standards

All animal experimental procedures were approved by the Ethics Committee of the Third Affiliated Hospital of Soochow University.

Results

Overexpression of TNFAIP3 ameliorates inflammation in the joints of mice with CIA

Arthritis was observed 28 days after collagen was injected into the mice. Compared with the control group, the TNFAIP3-oe group had lower arthritis scores (p = 0.030). H&E staining revealed that the mice in the control group presented severe synovitis and cartilage erosion. Most monocytes differentiate into macrophages in the synovium. Therefore, we detected macrophages in the joints through IHC. iNOS and CD206 were used to identify M1 and M2 macrophages, respectively. The positive cells in the three groups were compared through one-way ANOVA. **Figure 1** shows that more $iNOS^+$ cells were observed in the joints of the control mice ($p < 0.001$), whereas more $CD206^+$ cells were detected in the joints of the TNFAIP3-oe mice ($p < 0.001$), indicating that TNFAIP3 promoted M2 but not M1 differentiation.

TNFAIP3 inhibits the migration of monocytes

In the present study, *tnfaip3*-overexpressing or *tnfaip3*-depleted THP-1 cells were used to explore the function of TNFAIP3 in monocytes. Compared with those in the con-077 group, more cells in the TNFAIP3-i group migrated into the culture plate. Moreover, fewer cells migrated into the culture plate in the TNFAIP3-oe group than in the con-238 group. Furthermore, the expression levels of chemokine receptors and matrix metalloproteinases (MMPs) were compared via RT-PCR. Higher expression levels of CCR2 (p = 0.044), MMP2 (p = 0.039) and MMP3 (p = 0.040) were detected in the TNFAIP3-i group. MMP2 (p = 0.014) and MMP3 (p = 0.010) expression was lower in the TNFAIP3-oe group. However, there was no difference in the expression of CX3CR1 or MMP9 (**Figure 2**). These results suggest that monocytes expressing lower levels of TNFAIP3 have greater migration and that these cells can secrete more MMPs, which participate in cartilage erosion.

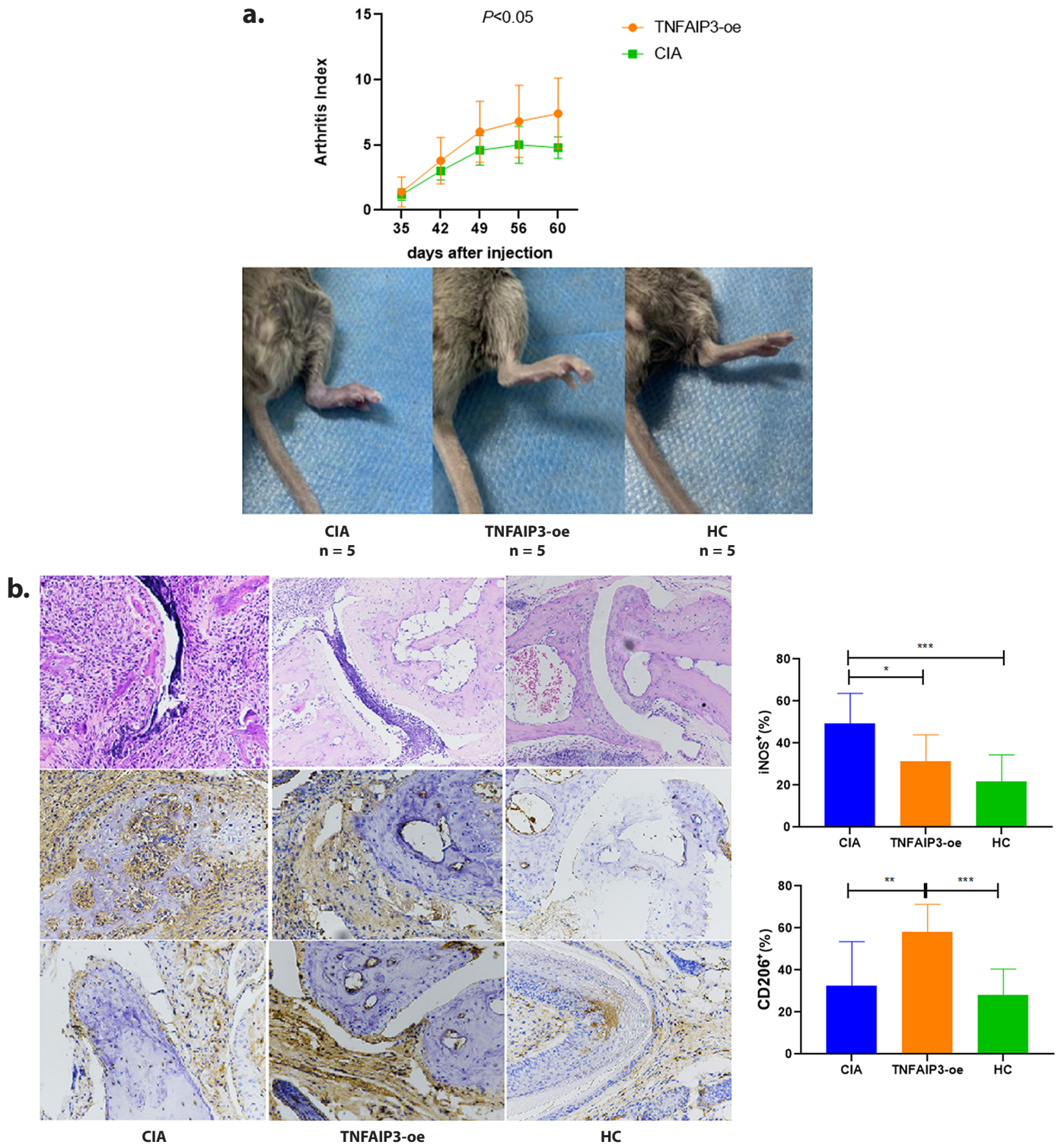

Figure 1. Overexpression of TNFAIP3 ameliorates inflammation in the joints of mice with collagen induced arthritis (CIA).
a. Arthritis in the mice with CIA and TNFAIP3-oe mice was compared through the arthritis index.
b. H&E staining and iNOS/CD206 expression in the joints of mice with CIA, TNFAIP3-oe mice and healthy control mice. Hyperplastic synovium was found in the mice from the CIA group and the TNFAIP3-oe group. In the hyperplastic synovium, $iNOS^+$ and $CD206^+$ cells were detected. Compared with those in the CIA group, lower iNOS and greater CD206 levels were detected in the TNFAIP3-oe group.

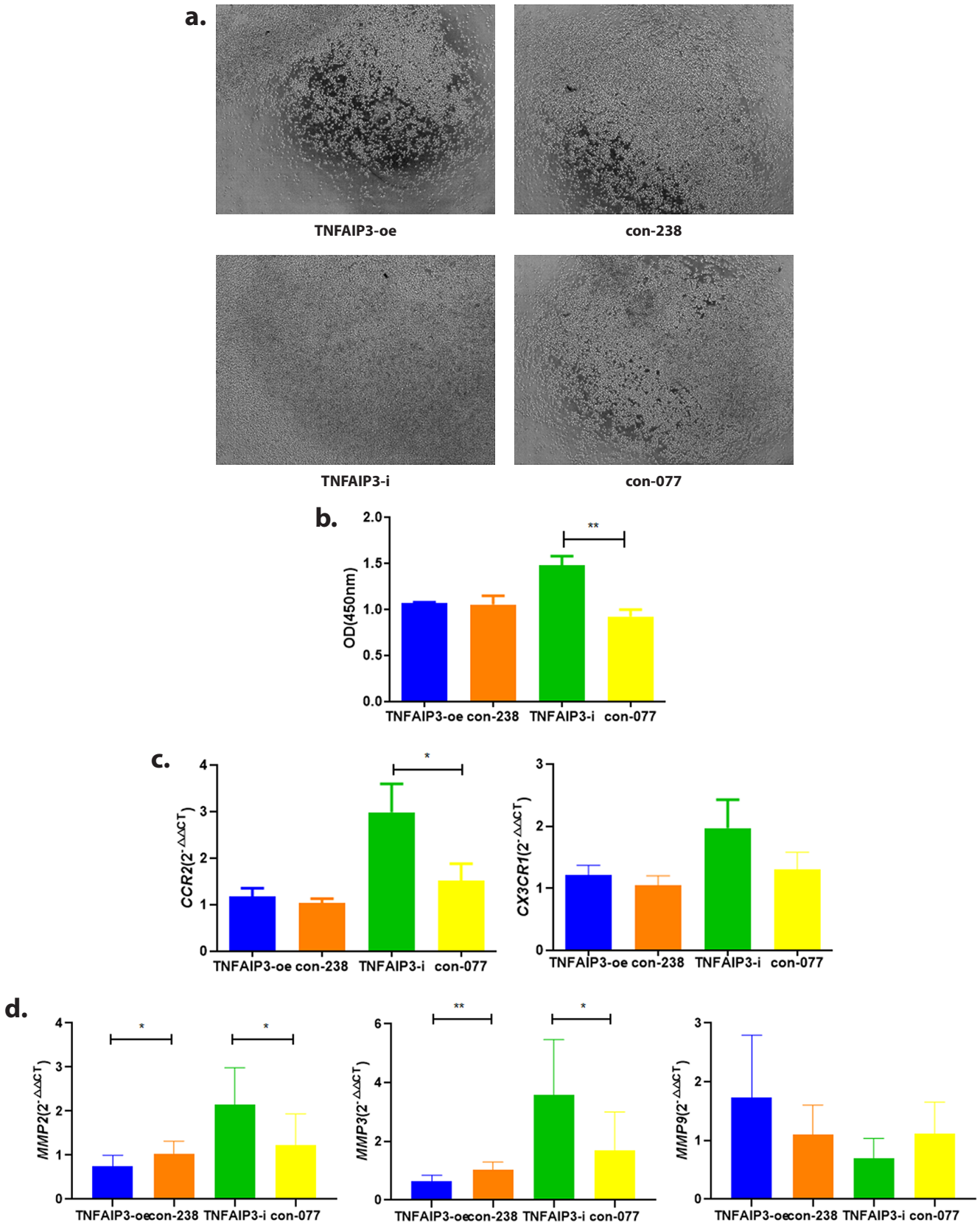

Figure 2. Comparison of the migration of THP-1 cells between the TNFAIP3-oe/TNFAIP3-i and control groups.
a. Optical microscopic photographs of cells in Transwell plates. The microscope magnification is 10 × 10.
b. The OD values of the plates were determined through a CCK-8 assay.
c. The expression of CCR2 and CX3CR1 in THP-1 cells was determined by RT-PCR.
d. The expression of MMP2, MMP3 and MMP9 in THP-1 cells was determined by RT-PCR.

TNFAIP3 inhibits the proinflammatory ability of monocytes

There are three subsets of monocytes, namely, CMs, IMs, and NCMs. Our previous study revealed that in RA, most CMs differentiate into IMs, which play a proinflammatory role. CMs differentiate into IMs by expressing less CD14 and more CD16.

Compared with the con-077 group, the TNFAIP3-i group expressed less CD14 and more CD16. This finding indicates increased differentiation into IMs by TNFAIP3 inhibition in monocytes. Furthermore, the TNFAIP3-i group secreted more of the proinflammatory cytokine IL-6, whereas no difference was detected in the secretion of TNF-α or IL-1β (**Figure 3**).

TNFAIP3 regulates the differentiation of monocytes

In the THP-1 culture system, PMA was added to induce monocyte differentiation into macrophages.

We observed different morphologies of macrophages from various groups via microscopy. For example, the TNFAIP3-oe cells were more circular in morphology, whereas the TNFAIP3-i cells had a more spindle-like morphology.

RT-PCR revealed that the expression of CD206 and CD163, which represent M2 macrophages, was greater in the TNFAIP3-oe group than in the other groups. Furthermore, the expression of IL-1β and IL-6, which indicates M1 macrophages, was lower in the TNFAIP3-oe group. These findings suggest that abnormal TNFAIP3 expression in monocytes affects subsequent macrophage polarization.

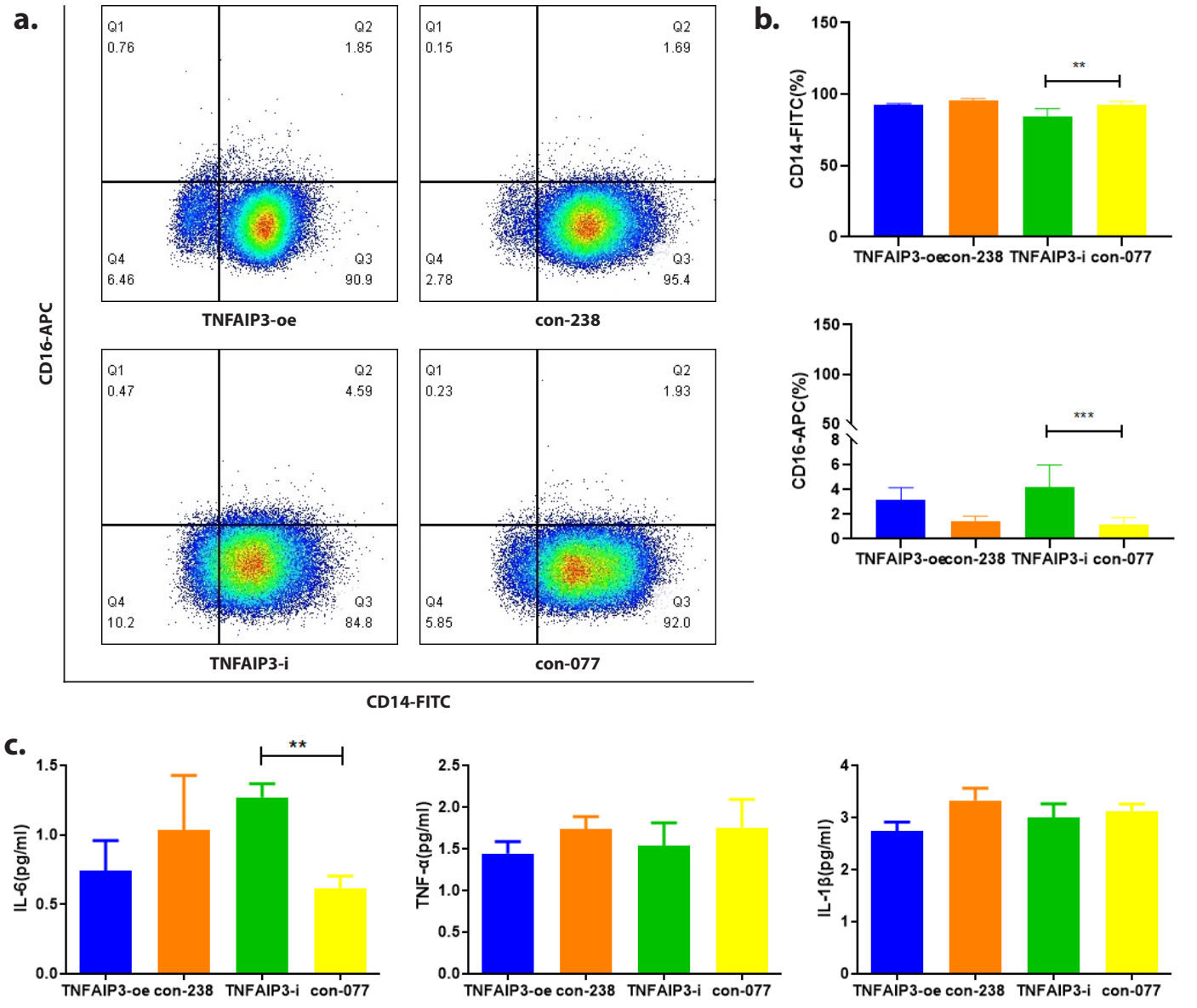

Figure 3. The proinflammatory role of THP-1 cells was compared between the TNFAIP3-oe/TNFAIP3-i and control groups.
a. The expression of CD14 and CD16 on the surface of THP-1 cells was detected by flow cytometry.
b. Comparison of CD14 and CD16 expression among the different groups.
c. The levels of proinflammatory cytokines in the THP-1 supernatants were compared by ELISAs.

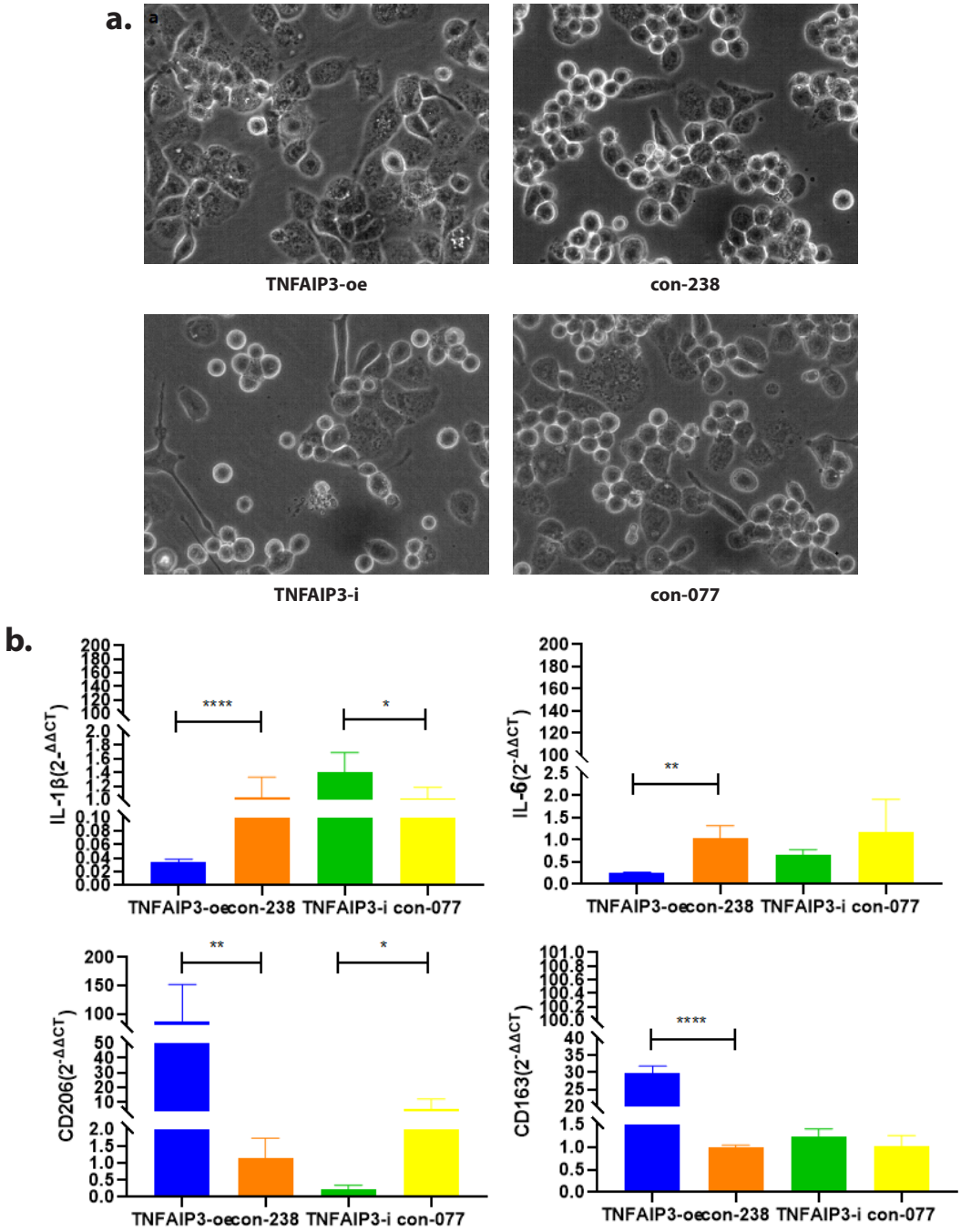

Figure 4. The ability of THP-1 cells to differentiate into M1/M2 macrophages is compared between the TNFAIP3-oe/ TNFAIP3-i and control groups.

a. Optical microscopic photographs of THP-1 cells after exposure to PMA. The microscope magnification is 10 × 40.

b. Comparison of M1/M2 marker expression among the different groups.

c. The levels of proinflammatory cytokines in the macrophage supernatants were compared by ELISAs.

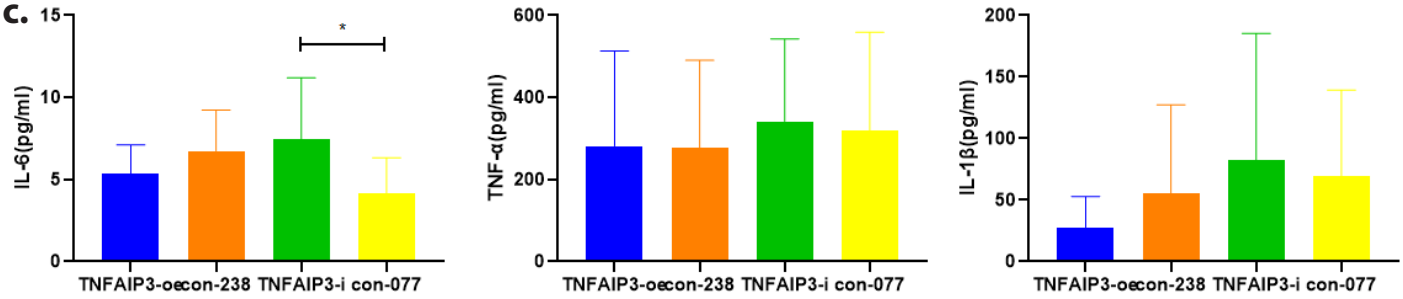

Figure 4. (Continued)

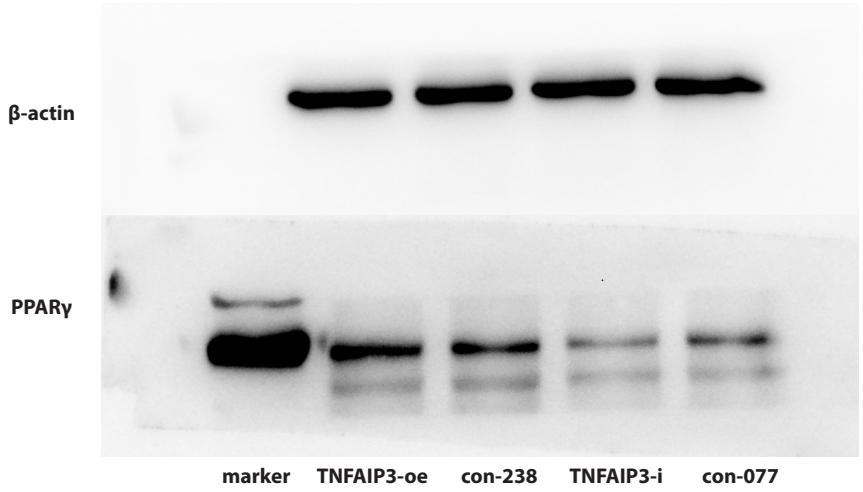

Figure 5. Activation of the cell signalling pathway in the differentiation of THP-1 cells into macrophages.
The PPARγ pathway was activated in TNFAIP3-oe macrophages and inhibited in the TNFAIP3-i macrophages.

The cells in the TNFAIP3-i group secreted increased levels of the proinflammatory cytokine IL-6 but not TNF-α or IL-1β (**Figure 4**).

PPARγ is activated during M2 polarization promoted by TNFAIP3

Previous studies have shown that TNFAIP3 inhibits inflammation by suppressing the NF-κB pathway. During the process of differentiation into M1 macrophages, the NF-κB signalling pathway is activated. Moreover, the PPARγ signalling pathway is activated during the process of differentiation into M2 macrophages.

We detected the expression of p-p65 in THP-1 cells and macrophages, as well as the expression of PPARγ in macrophages. No obvious difference in the expression of p-p65 was found between the TNFAIP3-oe or TNFAIP3-i groups and their control groups (con-238/con-077). In contrast, macrophages in the TNFAIP3-oe group expressed more PPARγ than those in the con-238 group did, and macrophages in the TNFAIP3-i group expressed less PPARγ than those in the con-077 group did (**Figure 5**).

Discussion

Synovitis plays a critical role in the pathogenesis of RA. Most macrophages promote synovitis by secreting proinflammatory cytokines and differentiating into osteoclasts in the RA synovium.[2,4] As one type of myeloid cell, tissue-resident macrophages can be differentiated from monocytes. Accordingly, we believe that more monocytes migrate into the synovium and differentiate into macrophages in RA. On the basis of this hypothesis, regulating monocyte migration could ameliorate RA.

In our previous study, we found more monocytes in the peripheral blood of RA patients, especially IMs and NCMs.[12] IMs are the primary proinflammatory monocytes that secrete proinflammatory cytokines and differentiate into macrophages.[7] Furthermore, we have shown that most IMs of RA patients express less A20.[12] These findings suggest that TNFAIP3 in monocytes plays a protective role in RA. To test our hypothesis, we injected lentiviruses overexpressing TNFAIP3 into CIA model mice. As expected, this treatment ameliorated synovitis in CIA, confirming the protective role of TNFAIP3. Moreover, the macrophage subtypes changed

after injection, indicating that TNFAIP3 might regulate macrophage polarization. However, as we used AAV vectors to upregulate TNFAIP3 expression in monocytes through tail vein injection, off-target effects might have occurred during this process. In addition to monocytes, other cells, such as synoviocytes, might be affected, which could regulate synovitis. Therefore, in our future studies, selected gene-modified mice would be a good choice.

Overall, tissue-resident macrophages contribute to tissue homeostasis and the resolution of inflammation.[13] Some tissue-resident macrophages are derived from peripheral blood monocytes. However, the migration of these cells partly depends on the need for effector cells during inflammation.[14] After they migrate to tissues, the phenotypes of macrophages can change under various conditions. Classically activated macrophages, known as M1 macrophages, play a proinflammatory role by secreting cytokines such as TNF-α and IL-6. In contrast, another phenotype, M2, is known as alternatively activated or healing macrophages.[15] In previous studies, some stimuli were reported to promote the expression of different genes, resulting in different macrophage phenotypes, and this process is called polarization. Th1 cytokines (TNF-α/IFN-γ) and LPS can promote macrophage polarization into the M1 phenotype, whereas Th2 cytokines (IL-4/IL-13) promote M2 polarization.[16] In the sublining of the RA synovium, more M1 markers but not M2 markers are detected.[17]

Cytokines or other stimuli were added to the culture system to promote macrophage polarization. In our *in vitro* study, we added only PMA, which helped THP-1 cells differentiate into naive macrophages (M0). However, the TNFAIP3-oe group expressed more M2 markers, and the TNFAIP3-i group expressed more M1 markers, which was consistent with their morphologies. In addition to cytokines, abnormal gene expression in monocytes, such as that of *tnfaip3*, can affect the polarization of macrophages.

TNFAIP3 is an anti-inflammatory factor that inhibits NF-κB activation. This molecule can inhibit NF-κB activation via multiple mechanisms. As NF-κB signalling cascades are heavily controlled by ubiquitination, TNFAIP3 inhibits this process by regulating protein ubiquitination. However, in our present study, NF-κB activation was not detected in the TNFAIP3-i group. In addition to NF-κB, other signal activators, such as STAT1 and IRF5, participate in the M1 pathway.[18] Further studies are needed to clarify how TNFAIP3 regulates the polarization of monocyte-derived macrophages to the M1 phenotype.

In the process of M2 polarization, many signalling pathways, including those involving IRF4, STAT6, and PPARγ, are activated.[19-21] He et al. combined bioinformatics and proteomics to identify the critical factors involved in M2 polarization and reported that MEK signalling is required for M2 polarization by PPARγ-induced retinoic acid signalling.[22] In the present study, we tested PPARγ and, surprisingly, detected its activation of M2 polarization in the TNFAIP3-oe group, preliminarily confirming that the activation of PPARγ in the process of M2 polarization is promoted by TNFAIP3. However, further inhibition experiments are needed to verify the regulatory ability of these compounds.

We showed that TNFAIP3 in monocytes regulated cell differentiation. In contrast, monocytes must migrate into the synovium before differentiating into synovial macrophages. Our present study revealed increased migration in the TNFAIP3-i group. The migration of cells depends on the combination of chemokines and chemokine receptors. Two widely demonstrated chemokine receptors on the monocyte surface are CCR2 and CX3CR1.[23] CCL2-CCR2 is one of the most critical axes in RA. One study has shown that the disease activity score of RA is associated with CCL2 levels.[24] Furthermore, CCL2 stimulates the differentiation and maturation of monocytes into macrophages and osteoclasts.[25] A previous study revealed increased levels of CX3CR1 in RA monocytes, especially IMs and NCMs.[26] Another study indicated that bone marrow-derived CX3CR1^{+} cells differentiate into osteoclasts in the inflamed synovium of mice with CIA.[27] Thus, CCR2 and CX3CR1 expressed on monocytes not only promote cell migration to the synovium but also participate in the inflammatory process of RA. Our study revealed increased migration and CCR2 expression in the TNFAIP3-i group. These findings suggested that TNFAIP3 in monocytes could regulate the expression of CCR2, affecting cell migration in RA.

After migrating to the synovium, monocytes can not only differentiate into macrophages but also play a proinflammatory role. Our previous study revealed that the number of monocytes, especially IMs and NCMs, is increased in the peripheral blood of RA patients and that monocytes promote inflammation through the secretion of cytokines, including TNF-α, IL-6, and IL-1β. However, in the present study, we detected greater IL-6 secretion in the TNFAIP3-i group than in the TNF-α or IL-1β group, indicating that TNFAIP3 in monocytes could inhibit inflammation by affecting IL-6 secretion. TNF-α and IL-6 are regarded as the central cytokines in RA. Previous studies have reported that TNFAIP3 can inhibit TNF-α by regulating NF-κB signalling.[28-30] However, the content of TNF-α in the culture supernatant of the TNFAIP3-i group remained unchanged in our study. IL-6 can regulate the differentiation of Th17, Treg, and Tfh cells, as well as osteoclastogenesis.[31,32] IL-6 can also upregulate the expression of VEGF, promoting angiogenesis in the RA synovium.[33] Clinically, the IL-6 antibody regimen has achieved excellent therapeutic effects. How TNFAIP3 in monocytes regulates the expression of IL-6 needs to be investigated in our future studies.

In the RA synovium, proinflammatory cells, such as synovial fibroblasts (FLSs), secrete MMPs,[34] including MMP2, MMP3, and MMP9, to invade cartilage. Not only FLSs but also monocytes can secrete MMPs. Moreover, the upregulation of MMPs promotes the migration and invasion of monocytes. In our present study, we found that the expression of MMP2 and MMP3 in the TNFAIP3-i group was increased, suggesting that TNFAIP3 could inhibit cartilage erosion by inhibiting the secretion of MMPs in monocytes.

In our previous study, we reported decreased expression of TNFAIP3 in the monocytes of RA patients. In this study, we demonstrated that abnormal expression of TNFAIP3 in monocytes could affect cell functions. However, in this study, THP-1 cells, not monocytes from RA patients, were used. Whether monocytes with lower TNFAIP3 expression from RA patients also have stronger proinflammatory functions and easier differentiation into M1 macrophages needs to be tested.

Furthermore, we demonstrated that PPARγ was activated in macrophages from the TNFAIP3-oe group but that NF-κB was not activated in macrophages from the TNFAIP3-i group, which was not consistent with the findings of other studies. Therefore, further research is needed to determine how the abnormal expression of TNFAIP3 in RA monocytes affects synovitis and its signalling pathway.

Conclusion

Overall, TNFAIP3 in monocytes can inhibit inflammatory arthritis by regulating macrophage differentiation and inhibiting cell migration and cytokine secretion. However, the underlying molecular mechanisms require further research.

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Disclosures

None.

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