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Review

# Effect of TNFα on osteoblastogenesis from mesenchymal stem cells

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ABSTRACT

*Background:* Bone destruction and osteoporosis are accelerated in chronic inflammatory diseases, such as rheumatoid arthritis (RA) and periodontitis, in which many studies have shown the proinflammatory cytokines, especially TNFα, play an important role; TNFα causes osteoclast-induced bone destruction as well as the inhibition of osteoblastogenesis.
*Scope of review:* Here we review our current understanding of the mechanism of the effect of TNFα on osteoblastogenesis from mesenchymal stem cells (MSCs). We also highlight the function of MSC in the pathogenesis of autoimmune diseases.
*Major conclusions:* Many studies have revealed that TNFα inhibits osteoblastogenesis through several mechanisms. On the other hand, it has been also reported that TNFα promotes osteoblastogenesis. These discrepancies may depend on the cellular types, the model animals, and the timing and duration of TNFα administration.
*General significance:* A full understanding of the role and function of TNFα on osteoblastogenesis from MSC may lead to targeted new therapies for chronic inflammation diseases, such as RA and periodontitis.
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## 1. Introduction

Bone mineral density and bone strength are mainly determined by the balance between bone formation by osteoblasts and bone resorption by osteoclasts. Interestingly, osteoblasts are derived from mesenchymal stem cells, whereas osteoclasts are derived from monocytes or macrophages of hematopoietic lineage. Osteoclastogenesis is under the strict control of osteoblasts producing macrophage colony-forming units (M-CSF), receptor activator of NFκB ligand (RANKL), and osteoprotegerin (OPG) [1]. However, recent studies have shown that osteoblasts regulate osteoclastogenesis through mechanisms independent of M-CSF, RANKL, and OPG [2].

Bone destruction and osteoporosis are accelerated in chronic inflammatory diseases, such as rheumatoid arthritis (RA) and periodontitis, in which many studies have shown the proinflammatory cytokines, especially TNFα, play an important role [3–5]; TNFα causes osteoclast-induced bone destruction [6–8] as well as the inhibition of osteoblastogenesis. In the current review article, we focused on the mechanism of the effect of TNFα on osteoblastogenesis from mesenchymal stem cells.

## 2. Mesenchymal stem cells

In 1976, Friedenstein et al. first identified bone marrow (BM) stromal cells, describing an adherent fibroblast-like population able to differentiate into the bone that they referred to as osteogenic precursor cells [9]. BM-derived mesenchymal stem cells (MSCs) reside in BM stroma, providing the supporting feeder cells necessary for hematopoietic progenitor cell growth but they may also differentiate into connective tissue cells, such as osteoblasts, osteocytes, chondrocytes, adipocytes and smooth muscle cells [10,11]. MSCs exist in almost all tissues; they can be easily isolated from the bone marrow, adipose tissue, umbilical cord, fetal liver, muscle, and lung, and can be successfully expanded in vitro [12]. In addition, in 2011, Kurth et al. reported the existence of resident MSCs in the knee joint synovium that undergo proliferation and chondrogenic differentiation following injury in vivo [13].

BMMSCs are thought to be derived from the bone marrow stromal compartment, initially appearing as adherent, single colony clusters (colony-forming unit-fibroblasts [CFU-Fs]), and subsequently proliferating on culture dishes [14]. To date, the CFU-F assay has been considered one of the gold standards for determining the incidence of clonogenic BMMSC [14]. In addition, a good correlation between CFU-F and MSC frequencies was obtained by flow cytometry using a previously published immunophenotype [15].

In 2006, the Tissue Stem Cell Committee of the International Society for Cellular Therapy proposed a set of minimum criteria that defined human MSC in the position paper [16] as follows. The cell must adhere to plastic when cultured under standard conditions and express the surface marker cluster of differentiation (CD) 73, CD90, and CD105, and not express CD45, CD34, CD14, CD11b, CD79, or CD19. Additionally, human MSC must be capable of in vitro differentiation into osteoblasts, adipocytes, and chondrocytes, i.e. "trilineage differentiation".

Platelet-derived growth factor receptor-α (PDGFR-α) + stem cell antigen-1 (Sca-1) + dual-positive (PαS) cells have been isolated and characterized in mice [17]. The PαS cells fulfill the basic requirements

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for the definition of MSC in mice. These cells are capable of unlimited self-renewal and can differentiate into osteoblasts, chondrocytes, and adipocytes under appropriate conditions in vitro [18]. On the other hand, CD146, a cell adhesion molecule of the immunoglobulin superfamily expressed in a restricted range of normal cells, is one such marker that has helped discern the in vivo localization and function of human MSC [19].

Due to the limitation of using embryonic stem (ES) and induced pluripotent stem (iPS) cells in the clinic, great interest has developed in MSC, which are free of both ethical concerns and teratome. MSCs have been shown to be effective in the treatment of many disorders, including both immune diseases and non-immune diseases. Osiris' Prochymal, the world's first stem cell drug approved in Canada on May 12, 2012, was successful in phase III clinical trials in treating graft versus host disease (GVHD) and Crohn's disease and has become the only stem cell-based drug approved by the FDA [12].

However, in 2011, MacDonald et al. highlighted that the findings from animal models of autoimmune rheumatic diseases may not be predictive of outcomes in clinical studies [20]. In addition, preclinical experimentation is a scientific and regulatory requirement, and therefore proper screening and selection of clinically relevant animal models that specifically reproduce the biologic features of the disease under study will be critical [20].

## 3. MSC in inflammatory-related bone diseases

Recently, a growing body of evidence has indicated that BMMSCs produce a variety of cytokines and display profound immunomodulatory properties, perhaps by inhibiting the proliferation and function of several major immune cells, such as natural killer cells, dendritic cells, and T and B lymphocytes [21].

### 3.1. Rheumatoid arthritis (RA)

RA is a chronic and systemic inflammatory disease, characterized by the destruction of the articular cartilage and bone in its chronic phase. Although histologic analyses of the periarticular trabecular bone have demonstrated that osteoclastic bone resorption is greatly stimulated in RA patients, the mechanism of the joint destruction in RA patients remains to be determined.

We have demonstrated that the number of CFU-F increases in the joints adjacent to the inflamed joints and correlates with the number of CFU-GM; the number of CFU-GM correlated with the level of IL-1β in the synovial fluid of the adjacent joint [22]. In addition, we have demonstrated the effect of bone marrow grafting on a titanium porous-coated implant in bilateral total knee arthroplasty [23], after we examined the characteristics of CFU-F of BM from patients with autologous bone grafting or rheumatoid arthritis (RA) [24,25]. The final fluoroscopically-guided radiographs revealed a decrease in the number of knees with radiolucent lines after marrow grafting compared to those without grafting, suggesting that the iliac bone marrow is useful as a bone grafting material to enhance biological fixation in porous-coated implants [23].

BMMSC may be primarily involved in joint damage in RA [26]. In addition, studies have demonstrated that increased local production of TNFα may injure the BM microenvironment and may affect the reserves of BM hematopoietic progenitor cells [27]. MSC from RA patients impaired clonogenic and proliferative potential in association with premature telomere length loss [28]. On the other hand, in patients with advanced osteoarthritis, chondrogenic and adipogenic activity of MSC were reduced [29].

### 3.2. Systemic lupus erythematosus (SLE)

SLE is a disease of unknown etiology in which tissues and cells are damaged by pathogenic autoantibodies and immune complexes. In 2007, Sun et al. reported that BMMSC derived from patients or mice with SLE showed impairment of osteogenic differentiation following successive cell passage in vitro [30]. On the basis of the promising clinical outcomes in SLE mice, Sun et al. treated four cyclophosphamide/glucocorticoid treatment-refractory SLE patients using allogenic bone marrow MSC transplantation (MSCT) and showed stable 12–18 months of disease remission in all treated patients [31]. The patients benefited from the amelioration of disease activity, and improvement in serologic markers and renal function, suggesting that allogenic MSCT may be a feasible and safe salvage therapy for refractory SLE patients [31]. In addition, because allogenic BMMSC from a patient's family member without human leukocyte antigen match is an easily accessible stem cell resource, MSCT may offer another effective cell therapy with fewer side effects [31].

### 3.3. Systemic sclerosis (SSc)

SSc is a chronic multisystem disorder of unknown etiology characterized clinically by thickening of the skin caused by accumulation of connective tissue and by involvement of visceral organ. Larghero et al. explored the phenotypical and functional characteristics of in vitro expanded bone marrow mesenchymal stem cells from patients with systemic sclerosis [32]. Their findings show that BM-derived MSCs from patients with SSc under the described culture conditions exhibit the same phenotypic, proliferative, differentiation potential and immunosuppressive properties as their healthy counterparts.

## 4. Mechanisms of osteoblast differentiation

### 4.1. Role of Wnt in osteoblastogenesis

Osteoblasts are derived from mesenchymal progenitor cells in the bone marrow or pericytes. Their maturation process includes consecutive stages of proliferation, matrix production and matrix mineralization [39]. Osteoblasts can ultimately become osteocytes. Activation of the Wingless-type MMTV integration site (Wnt) pathways facilitates osteoblast specification from mesenchymal progenitors and enhances bone mass and strength. Thus, the Wnt pathway has emerged as a crucial regulator of bone formation and regeneration (Fig. 1). For more details of Wnt signaling, we recommend the review article of Kuhl et al. [40].

### 4.2. Effect of GSK3β on osteogenesis

Glycogen synthase kinase 3β (GSK3β) is known to modulate cell apoptosis and differentiation through multiple intracellular signaling pathways [41]. GSK3 is now known to target multiple cell regulatory proteins and to be controlled by both Wnt signaling and the PI3K/Akt

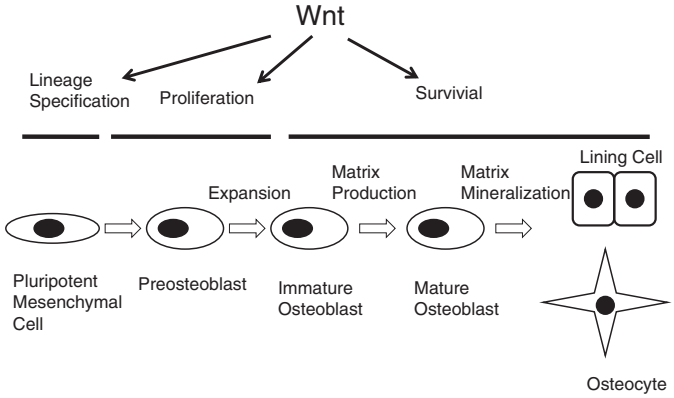


**Fig. 1.** Wnts affect multiple stages of osteoblast-lineage maturation. Modified from ref. [39].

pathway. In addition to β-catenin, the targets of GSK3 that have been implicated in the regulation of cell proliferation and differentiation include several transcription factors of NFκB signaling. Recently, some studies have found that GSK3β could modulate both NFκB and β-catenin activities through phosphorylation or transcriptional regulation. However, it was not clear whether GSK3β regulates NFκB and β-catenin activities in inflammation. In 2013, Chen et al. reported that NFκB modulates the osteogenesis of periodontal ligament stem cells (PDLSCs) through competition with β-catenin signaling in inflammatory microenvironments [41]. Their data indicate that NFκB has a central role in regulating osteogenic differentiation of PDLSCs in inflammatory microenvironments. Given the molecular mechanisms of NFκB in osteogenic differentiation governed by inflammation, it can be said that NFκB helps to improve stem cell-mediated inflammatory bone disease therapy.

## 5. Stimulation of TNFα production on osteoblastogenesis in diseases

### 5.1. RA and postmenopausal osteoporosis

The majority of studies of anti-TNFα therapies have focused on RA, and the positive effects of these therapies have been considered secondary to mitigation of the chronic inflammatory nature of RA. On the other hand, studies have shown that TNFα plays a central role in the pathophysiology of postmenopausal osteoporosis [33]. It is speculated that TNFα inhibits osteoblastogenesis by the mechanism as mentioned in Section 6. Although postmenopausal osteoporosis is a chronic inflammatory environment, the microenvironment in postmenopausal osteoporosis is very different from RA; unlike RA, estrogen deficiency plays a role in postmenopausal osteoporosis, which could impair stem cell activity [34].

### 5.2. Fracture

TNFα is a major inflammatory factor peaking 24 h after bone fracture in response to injury; its role in bone healing is controversial. It has been reported that short-term exposure to TNFα enables human osteoblasts to direct adipose tissue-derived MSC into osteogenic differentiation [35]. On the other hand, it has been reported that TNFα promotes fracture repair by augmenting the recruitment and differentiation of resident stromal cells present in the muscle [36]. Molecular analysis of human fracture samples revealed that TNFα produced at the fracture site can (at the appropriate concentration) recruit stem cells and promote repair in mouse models [36].

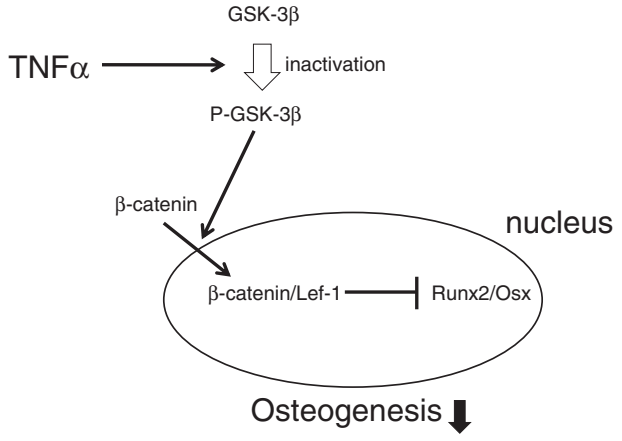


**Fig. 2.** GSD3β as a checkpoint for TNFα-mediated impaired osteogenic differentiation of MSC in inflammatory microenvironments. In inflammatory microenvironments, TNFα induced the phosphorylation of GSK3β, and the p-GSK3β subsequently resulted in nuclear β-catenin accumulation and β-catenin/Lef-1 complex formation, which inhibited Runx2-associated osteogenesis of PDLSCs [7].

### 5.3. Inflamm-aging

In the clinical population, TNFα, interleukin 6 (IL-6), and IL-1 have been consistently demonstrated to be increased in serum in the aged population. Thus, Franceschi et al. have characterized aging as "inflamm-aging," that is, an age-associated inflammatory status [37]. Wahl et al. used a unique model of bone regeneration to demonstrate (a) that age-related deficits in direct bone formation can be restored to young mice by treatment with TNF blockers and (b) that the cyclin-dependent kinase inhibitor p21 is a candidate for mediation of the osteoinhibitory effects of TNF [38].

## 6. TNF inhibits osteoblastogenesis

Previous studies have demonstrated the ability of TNFα to inhibit multiple osteoblast functions in vitro as well as fracture repair in vivo [42]. The signal-transduction pathways activated by TNFα binding to its receptors have been studied extensively in several systems [43]. In regard to TNFα effects on osteoblastogenesis in vitro, recent work using fetal rat calvarial cells and a murine calvarial osteoblastic cell line has demonstrated that TNFα (a) is a potent inhibitor of osteoblast differentiation from precursor cells, (b) acts distal to insulin-like growth factor I (IGF-I) and bone morphogenetic proteins (BMPs), (c) inhibits the expression of RUNX2 and osteoblast-associated transcription factors (Osterix) through MEK1 kinases, (d) suppresses vitamin D-stimulated transcription owing to the activation of transcription factor NFκB, and (e) actions are mediated by TNFR1 [44]. Although high levels of TNFα are known to inhibit direct bone formation in culture and in vivo, nevertheless, low doses can enhance osteoblast proliferation in culture, and impaired bone formation has been demonstrated in TNFR1/R2 double-knockout mice [43,44]. This suggests that a homeostatic level of TNFα signaling is required for optimal bone formation but that unregulated or excessive expression results in pathology.

## 7. The inhibitory effect of TNFα on osteogenesis

### 7.1. GSK3β is a checkpoint for TNFα-mediated impaired osteogenic differentiation of PDLSC

In the recent issue of BBA, Kong et al. reported that GSK3β is a checkpoint for TNFα-mediated impaired osteogenic differentiation of MSC in inflammatory microenvironments [5] (Fig. 2). Their findings demonstrated that in inflammatory microenvironments, TNFα induced the phosphorylation of GSK3β, and p-GSK3β subsequently resulted in nuclear β-catenin accumulation and β-catenin/Lef-1complex formation, which inhibited Runx2-associated osteogenesis of human periodontal ligament tissue-derived mesenchymal stem cells (PDLSCs), which are organ-specific MSC. It was shown that GSK3β was required for TNFα-mediated inhibition of osteogenic differentiation in MSC, which provided new insight into the functional role of GSK3β. A strategy to target GSK3β may provide a novel potential approach to bone regeneration in inflammatory microenvironments.

### 7.2. MicroRNA17 (miR-17) in PDLSC

MicroRNAs (miRNAs) have emerged as key negative small molecular regulators of gene expression. miR-17 is encoded by the miR-17–92 cluster, which comprises seven mature miRNAs (miR-17, miR-17-3p, miR-18a, miR-19a, miR-20a, miR-19b, and miR-92a) [45]. It is suggested that miR-17 has a critical role in the regulation of cell proliferation and differentiation as well as in the control of various processes involved in maintaining health and disease [46]. Liu et al. demonstrated using human PDLSC that inflammation in the microenvironment promoted

the expression of Smad ubiquitin regulatory factor one (Smurf1), an important negative regulator of MSC osteogenic differentiation [34]. In addition, they showed that excessive inflammatory cytokine levels, miR-17, and Smurf1 were all involved in a coherent feed-forward loop. In this circuit, cytokines led to direct activation of Smurf1 and downregulation of miR-17, thereby increasing the degradation of Smurf1-mediated osteoblast-specific factors [46] (Fig. 3A).

In addition, Liu et al. reported interesting findings in 2013: a canonical Wnt signal acts as a positive or negative regulator of PDLSC' osteogenesis in different microenvironments depending on the expression level of miR-17 [47], because miRNAs were more sensitive and responded more rapidly to the microenvironment than the signaling complex. As mentioned above, Liu et al. confirmed that the function of miR-17 changed depending on the microenvironment [46]. Thus, it was deduced that miR-17 acted as a 'toggle switch' in modulating signaling pathways, suggesting that this may have applications in miRNA regulation of so far enigmatic, and necessarily holistic, aspects of biological processes, such as differentiation, homeostasis and regeneration [47].

In 2013, in addition, the same group reported interesting findings using ovariectomized (OVX) mice and BMMSC from estrogen deficiency-induced osteoporosis patients [34]. As mentioned in Section 5.1, estrogen deficiency-induced osteoporosis is caused by elevated inflammatory cytokines, especially TNFα. miR-21 was suppressed by TNFα in the impaired osteogenic differentiation of MSC in estrogen deficiency-induced osteoporosis patients. miR-21 promoted MSC osteogenesis by repressing its target gene, Spry1. Blocking TNFα in OVX mice promoted bone formation by activating the miR-21–Spry1 functional axis (Fig. 3B). These findings introduce a novel mechanism in which TNFα impairs osteoblastic bone formation by suppressing miR-21 expression in estrogen deficiency-induced osteoporosis.

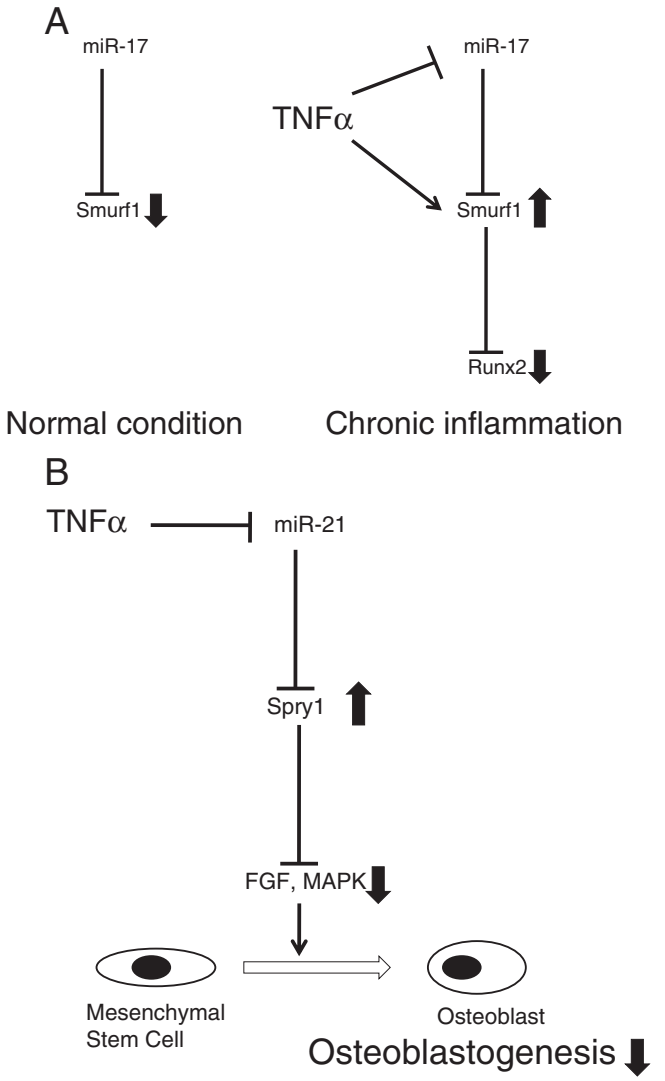


**Fig. 3.** Effect of TNFα on miRs. A. Working model of miR-17 in a coherent feed-forward loop. (Left): In the absence of TNFα signaling, the expression of the target Smurf1 is repressed by miR-17 and proper ubiquitination is maintained. (Right): In the presence of TNFα signaling, the target can be activated directly, but it can also be activated through inhibition of miR-17, thereby leading to coherent regulation. Abbreviations: Smurf1, Smad ubiquitin regulatory factor one; TNF, tumor necrosis factor. B. A hypothetical diagram of the axis of TNFα–miR-21–Spry1 regulation in MSC of osteogenic differentiation. miR-21 promotes MSC osteogenesis by repressing its target gene, Spry1. Blocking TNFα in OVX mice promotes bone formation by activating the miR-21–Spry1 functional axis. Spry1 is a negative regulator of the FGF and ERK–MAPK signaling pathways, which are established as being involved in promoting MSC osteogenesis.
Panel A is modified from ref. [46] and panel B is modified from ref. [34].

## 8. Stimulatory effect of TNFα on osteogenesis

Hess et al. showed that TNFα increases BMP-2 expression in hMSC through the NFκB signaling pathway in early osteogenic differentiation [48]. NFκB stimulates critical regulators of osteogenesis such as BMP2, RUNX2, and Osterix and these events finally result in enhanced mineralization of the extracellular matrix.

Several earlier reports had suggested that TNFα is a negative regulator of osteoblast differentiation, as mentioned in Section 6. An important difference between the earlier studies and the approach of Hess et al. lies in the differentiation state of the starting cells. Hess et al. used multipotent human MSC to study early osteoblastogenesis. These cells are able to differentiate into several mesenchymal lineages and therefore allow them to analyze molecular decisions during the first steps of lineage commitment.

Interestingly, Huang et al. reported dose-specific effects of TNFα on osteogenic differentiation of MSC [49]. Levels of Runx2, Osx, OC and ALP were up-regulated in cell cultures treated with TNFα at lower concentrations, while down-regulated in cell cultures treated with TNFα at higher concentrations. Blockade of NFκB signaling reversed the inhibitory effect observed in cell cultures treated with TNFα at higher concentrations, but showed no effect on cell cultures treated with TNFα at lower concentrations. These findings suggested that the binding of TNFα to its receptors results in the activation of multiple signaling pathways [49].

In addition, Briolay et al. reported autocrine stimulation of osteoblast activity by Wnt5a in response to TNFα in human mesenchymal stem cells [50]. Although TNFα treatments efficiently block inflammation in ankylosing spondylitis (AS), they are inefficient to prevent excessive bone formation. In AS, ossification seems more prone to develop at sites where inflammation has resolved following anti-TNF therapy, suggesting that TNFα indirectly stimulates ossification. Wnt5a may be potentially involved in the effects of inflammation on bone formation, acting through non-canonical signaling. Wnt5a may therefore represent a possible target to prevent syndesmophyte formation in AS.

## 9. Conclusions

Many studies have revealed that TNFα inhibits osteoblastogenesis through several mechanisms. On the other hand, it has been also reported that TNFα promotes osteoblastogenesis. These discrepancies may depend on the cellular types, the model animals, and the timing and duration of TNFα administration. To develop more effective therapies targeting TNFα, these mechanisms remain to be elucidated.

## Conflict of interest

The authors declare that they have no conflicts of interest.

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