

The Role of TNF- α in Rheumatoid Arthritis

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Abstract. Rheumatoid arthritis (RA) induces articular damage through autoimmunity. Tumor necrosis factor- α (TNF- α) plays an important role in the pathogenesis of RA. TNF- α binds to its receptors to recruit certain molecules and forms two kinds of complexes (complex I and II) to regulate the pro-survival, apoptosis, and necrosis of cells. TNF- α upregulates RA by the methylation and acetylation of the RA synovial fibroblast gene and causes RA-derived joint damage through the induction of osteoclasts. Two TNF- α receptors mediate RA in opposite directions. TNF- α has been a target for the treatment of RA in which TNF- α inhibitors (TNFi) are administered periodically for RA patients. Several mechanisms in the RA-related TNF- α signaling pathways remain imprecise including the regulation of regulatory T cells, osteoclast induction, and the potential interaction with the hypoxia-inducible factor. Further investigation into these questions may point out new methods for RA treatment to overcome the defects of current TNFi such as the anti-drug antibodies.

Keywords: Rheumatoid Arthritis, Tumor Necrosis Factor- α , Articular Damage, TNF- α Receptor, TNF- α Inhibitor.

1. Introduction

1.1. Rheumatoid arthritis in articular damage

Besides injuries caused by sports, human joints can be damaged by diseases. Hyaline cartilage lowers the friction coefficient between the joints, providing lubrication to protect the joints from abrasion. Diseases including osteochondritis dissecans, relapsing polychondritis, chondrocalcinosis, and inflammatory arthropathies may damage the cartilage between the joints. Rheumatoid arthritis (RA) induces autoimmunity among inflammatory arthropathies and chronically affects the cartilage and bone in the joints by inflammation. Autoreactive immune cells lead to an inflammatory cascade which causes synovial hyperplasia and fibrinous deposits. Many parts of the joints are targeted by inflammation such as the articular cartilage, the bone at the margins of the joint, and periarticular and subchondral bones. RA patients show three forms of bone loss which are localized bone erosion, periarticular osteopenia, and generalized osteoporosis. The margins of joints where inflamed synovium directly contacts the bones are the sites of RA erosion, which has a distinct demarcation when compared to bone loss in other inflammatory rheumatic disorders such as spondylarthritis. Most bone loss of RA radially locates in metacarpophalangeal or metatarsophalangeal and proximal interphalangeal joints. Radiological signs of periarticular osteopenia are commonly found in RA patients. Compared with the general population, RA also leads to secondary systematic osteoporosis which increased in the femoral neck, the total hip, and the spine in RA patients. Osteoclasts which are a kind of multinucleated cells contribute to bone resorption. Analysis of the samples obtained from RA patients after total joint replacement arthroplasty showed that the osteoclasts locate in resorption lacunae in calcified cartilage and its subchondral bone [1]. In mice lacking the nuclear factor- κ B ligand-receptor activator (RANKL), osteoclasts were absent, and there was less bone degradation. No histological evidence of articular bone erosion was discovered in c-Fos defective mice, even though these animals did not produce osteoclasts and suffered from arthritis through the production of human tumor necrosis factor.

1.2. Cytokines related to RA

TNF- α and interleukin (IL) are cytokines that are linked to RA. TNF- α and IL-6 are two key regulators of RA pathogenesis. TNF- α engages in multiple directions of the signaling pathway in RA.

In the synovium during RA inflammation, B cells and synovial fibroblasts secrete IL-6, which helps to produce immunoglobulin. IL-6 has three kinds of initiation of the signaling pathway with its receptor. The first takes place in myeloid cells and lymphocytes when IL-6 binds to the IL-6 receptor α (IL-6R α), which causes glycoprotein 130 (gp130) to dimerize and activate intracellular signaling. The second occurs in synovial fibroblasts and endothelial cells in which IL-6 links to the secreted form of IL-6R α (sIL-6R α) and forms a complex with gp130. The third involves IL-6 binding to its receptor and the formation of the complex in dendritic cells. The complex on the dendritic cell then binds to gp130 on the CD4⁺ cell. The gp130 homodimer is also generated by the complex including IL-6, IL-6R, and gp130 that is formed in the second and third kinds. Gp130 dimerization activates the Janus kinase (JAK) which leads to the STAT signaling. The human T helper 17 (Th17) cell transcriptional regulation throughout the early stages of development depends on the IL-6/STAT3 pathway. IL-6 produced by synovial fibroblasts under autoimmune arthritis also helps convert Forkhead box p3 (Foxp3)-positive T cells to Th17 cells which are more potential for the induction of osteoclasts compared with naïve Th17 cells generated by CD4⁺ T cells. The IL-17 cytokine family promotes neutrophil recruitment and activation, fibroblast-like synoviocyte activation, and the induction of chemotaxis in inflamed areas. Osteoclast formation is mediated by RANKL, which is activated by both IL-6 and IL-17. RANKL is expressed by fibroblast-like synoviocytes from RA patients (RA-FLS) when IL-6 is present. In fibroblast-like synoviocytes in vitro, RANKL was induced by IL-17 from the rat with adjuvant-induced arthritis. IL-23 mediates the conversion from $\alpha\beta$ T CD4⁺ naïve cells into Th17 cells and helps induce other proinflammatory cytokines from dendritic cells, Th17 cells, and macrophages. IL-23 receptor α also promotes the development of Th17 cells through JAK/STAT signaling pathway. IL-1 β can cause inflammation, damage of cartilage, and bone resorption by its mediation in the induction of macrophages and fibroblasts, the activation of chondrocytes, and the activation of osteoclasts separately. In RA, the beginning and continuation of the inflammatory response are intimately linked to IL-18. Pro-inflammatory cytokines are produced and released as a result of signaling pathways that are related to IL-33. Through an antigen-presenting cell's contribution to the IL-6 and IL-23 synthesis, granulocyte macrophage colony-stimulating factor (GM-CSF) contributes to Th17 which then creates GM-CSF and completes the positive-feedback cycle, which induces inflammation.

This review focuses on TNF- α 's role in RA, including TNF- α 's downstream signaling, TNF- α 's role in RA pathogenesis, and TNF- α 's current clinical inhibitors for RA.

2. TNF- α in RA

2.1. Introduction of TNF- α

TNF- α was first demonstrated to cause tumor necrosis compared with endotoxin in bacillus Calmette-Guérin-infected mice [2]. TNF- α was first isolated from HL-60 cell cultures stimulated by the tumor-promoting agent 4 β -phorbol 12 β -myristate 13 α -acetate (PMA) and identified in cDNA and protein levels. Its amino acid sequence showed about 30% similarity to lymphotoxin (TNF- β). X-ray crystallography determined TNF- α as a trimer at 2.6 Å resolution and each of its monomer shapes as a slight antiparallel β -sandwich. Some chronic inflammatory disorders are brought on by the pro-inflammatory cytokine TNF- α . The precursor transmembrane form (mTNF- α) and the soluble form (sTNF- α) are the two different forms of TNF- α . The transmembrane form functions more in the contacts between cells while soluble TNF- α functions in systematic activity far from the cells secreting it. The TNF- α -converting enzyme can be activated and transform the transmembrane TNF- α to the soluble form under cytokine stimulation.

2.2. TNF- α signaling pathways

Both mTNF- α and sTNF- α regulate downstream pathways through tumor necrosis factor receptor (TNFR) 1 and 2. TNFR1 is expressed in all types of cells while TNFR2 is mainly expressed in endothelial, immune, and neural cells. Human TNFR1 and TNFR2 share 28% similarity in their

extracellular domains but do not show homology in their intracellular domains. Because TNFR1 has a death domain (DD) in its intracellular structure, it can attract TNFR1-associated DD (TRADD) through TRADD's C-terminal and control programmed cell death. TNFR2, on the other hand, lacks a DD and instead activates TNFR-associated factor (TRAF) via the RING finger domain.

When TNF- α binds to TNFR1, TNFR1 transforms its DD to recruit TRADD. TRADD can form two kinds of complexes among which complex I is associated with cell pro-survival signaling pathway and complex II induces apoptosis and necrosis of cells. The complexes signaling requires the ubiquitination of RIPK1. Both ends of ubiquitin contain lysine sites.

TRADD joins up with TRAF 2/5, cIAP 1/2, RIPK1, and the linear ubiquitin chain assembly complex (LUBAC) to produce complex I. LUBAC leads to the induction of the TAK1 complex and the inhibitor of κ B (IkB) kinase complex by ubiquitinating RIPK1 with Met1-linked polyubiquitin chains. The TAK1 complex phosphorylates the mitogen-activated protein kinase (MAPK), which then phosphorylates Jun N-terminal kinase (JNK), which activates p38. The TAK1/MAPK/JNK/p38 cascade translocates activating protein 1 (AP1) to the nuclear. Besides Met-1 linked ubiquitin, cIAP adds K63-linked polyubiquitin chains to complex I. Two types of ubiquitin help phosphorylation of the inhibitor of κ B kinase (IKK) complex by TAK1 complex. The IKK complex consists of IKK α , IKK β , and IKK γ . Activated IKK β activates the nuclear factor kappa B (NF- κ B) pathway by degrading the inhibitor of κ B α (IkB α) and translocating NF- κ B to the nucleus [3]. Cell survival is initiated by the translocation of AP1 and NF-B, which inhibits inflammation and cell proliferation.

TRADD forms complex IIa with a homodimer of pro-caspase 8 and FAS-associated DD (FADD) which is a heterodimer of cFLIP_L and pro-caspase 8. Although RIPK1 takes part in the complex, it is inactivated by the heterodimer to prevent necrosis. If the RIPK1-induced activation of NF- κ B is interfered by cylindromatosis (CYLD) which damage K63-ubiquitin-conjugated moieties linked with RIPK1, cFLIP_L will not translocate to complex IIa to prevent the autoactivation of pro-caspase 8 [4, 5]. Then the pro-caspase begins autocatalytic cleavage and activates caspase 8 which induces the initiation of cell apoptosis [6]. Although a low level of cFLIP_L prevents the cleavage activity of the pro-caspase-8-cFLIP_L heterodimer on RIPK1, the active caspase 8 can also inactivate RIPK1 to remain apoptosis [7].

Non-ubiquitylated RIPK1 leads to complex IIb which shows the effect of upstream disruption compared to the interference with the canonical NF- κ B pathway. The mitochondrial protein Smac/DIABLO can deplete cIAP 1/2 which separates RIPK1 from the complex I [8]. The non-ubiquitylated RIPK1 then forms complex IIb (ripoptosome) with RIPK3, cFLIP_L, and pro-caspase 8 after degradation of cIAP 1/2 in the early stage of the pathway [9]. Then complex IIb induces cell apoptosis under a low cFLIP_L level in a similar way to the complex IIa pathway [10]. If neither RIPK 1 nor 3 are deactivated, they will join forces to create complex IIc, which phosphorylates the protein with the mixed lineage kinase domain (MLKL) [11].

TNFR2 directly binds to TRAF 1/2 to recruit cIAP 1/2 which induces complex I. Besides AP1-related cell survival and canonical NF- κ B pathway, TNFR2 also activates NF- κ B inductor kinase (NIK) which regulates the phosphatidylinositol 3-kinase (PI3K) pathway to upregulate neuron survival through noncanonical NF- κ B pathway [12]. The binding capacity of TNFR2 with TRAF2 is weaker than that of TNFR1 probably resulting from the novel C-terminal binding site of TNFR2 which shows attenuation of the activation of TRAF2. The 343-349 amino acid fragments of TNFR2 contribute to the degradation of TRAF2 which downregulates the activation of the NF- κ B pathway [13]. Therefore, TNFR2 may autoregulate its signaling by regulating the intracellular concentration of TRAF2.

2.3. TNF- α signaling in RA

RA patients show more expression of TNF- α . TNF- α plays multiple roles in RA pathogenesis including activation of endothelial cells, recruitment of proinflammatory cells, regulation of T cells, and growth of osteoclasts. Global single-cell transcriptomic profiling shows that multiple monocyte, B cell, and T cell populations produce most TNF- α in synovia with RA. Patients with RA produce

synovial macrophages similar to murine extrinsic macrophages which express TNF- α . Anti-cyclic citrullinated peptide antibody (ACPA) indicates the development of bone injury in RA. By creating immune complexes that drive macrophages to generate TNF-, ACPA can cause RA [14]. TNF- α produced by different cells regulates RA in different ways. B cell-produced TNF- α regulates the generation of pathogenic autoantibodies, which in turn determines the severity of RA. T cell-derived TNF- α shows a protective effect in the induction of RA by regulating the development of autoreactive memory T cells after dendritic cells limit the production of interleukin-12. Myeloid cell-derived TNF- α activates the synovial fibroblasts and regulates both the induction and pathogenesis stages of RA [15].

At the epigenetic level, TNF- α regulates the methylation and acetylation of the RA synovial fibroblast gene. TNF- α reduces methylation by suppressing DNA methyltransferase (DNMT). This promotes the production of pathogenetic genes like matrix metalloproteinase-19 (MMP-19), C-X-C motif chemokine ligand 8, and interleukin-6 (IL-6) (CXCL8). In RA, TNF- α selectively suppresses histone deacetylases 5 (HDAC5) in fibroblast-like synoviocytes (FLS), reduces inflammation by decreasing acetylation, and induces inflammatory cytokines and chemokines [16,17].

TNF- α causes RA bone damage by inducing the differentiation of osteoclasts. Differentiation of osteoclast is usually caused by the comparison between the RANKL and osteoprotegerin (OPG). OPG can bind RANKL to block the interaction between the receptor activator of NF- κ B (RANK) and RANKL. Downregulation of RANK-RANKL interaction prevents the differentiation of osteoclasts and protects bones from resorption by osteoclasts [18]. However, denosumab which is a kind of RANKL inhibitor fails to narrow the space between joints in RA and regulate the activity of RA in clinical trials, which indicates other pathways may induce osteoclast differentiation [19]. TNF- α is thought to induce osteoclast similar to the RANKL/OPG signaling pathway. Evidence suggests that TNF- upregulates the nuclear factor of activated T cells 1 (NFATc1), NF-B p50/p52, and c-Fos cascade, which produces osteoclasts [20]. The induction of osteoclasts by TNF- α can be amplified by IL-1 which is produced by osteoclast precursors (OCPs) and shows autocrine function after the upregulation of c-Fos [21].

TNFR 1 and 2 play opposite roles in RA. In a collagen-induced arthritis (CIA) model, knocking out TNFR1 downregulates CIA activity and the antagonist of TNFR1 also attenuates the arthritis. On the contrary, TNFR2-knockout mice suffer from harsh arthritis while the agonist of TNFR2 reduces the severity of CIA [22–25]. These results show that the TNFR1 signaling pathway tends to upregulate the inflammatory target genes while the TNFR2 signaling pathway downregulates inflammation. Both TNFR 1 and 2 are associated with regulatory T (T_{reg}) cells in RA. Immune homeostasis is preserved and autoimmune disease is prevented by T_{reg} cells. Foxp3 controls the differentiation of T_{reg} cells. Depletion of TNFR1 elevates the activity of T_{reg} cells. Because TNFR1 signaling induces the NF- κ B pathway which initiates the transcription of protein phosphatase 1 (PP1). PP1 will deactivate Foxp3 by dephosphorylating the Ser418 site and make Foxp3 unable to bind the target sequence and prevent the differentiation of T_{reg} cells. TNF- α -derived dysfunction of T_{reg} cells shows a correlation with the elevated number of IL-17⁺ and interferon- γ ⁺CD4⁺ T cells, which displays the balancing function of TNF- α between protective and pathogenic T cells [26]. TNFR2 signaling is suggested to promote the development and functional activity of T_{reg} cells. Lack of TNFR2 in inflammatory circumstances causes more severe arthritis, fewer T_{reg} cells, and the loss of the inhibitory function of T_{reg} cells. In addition, without TNFR2, T_{reg} cells tend to differentiate to a proinflammatory phenotype similar to Th17 [23]. In experimental arthritis models, the transfer of T_{reg} cells from wild-type (WT) mice to TNFR2^{-/-} mice protects the TNFR2-deficient mice from aggravation of arthritis [27].

2.4. Clinical TNF- α inhibitors in RA

Three types of TNF- α inhibitors (TNFi) are employed in clinical RA treatments: monoclonal antibodies (infliximab, adalimumab, and golimumab), TNF receptor constructs (etanercept), and antibody fragments (certolizumab pegol). Etanercept is the first cytokine inhibitor approved for RA

by Food and Drug Administration (FDA) in the U.S.A. Etanercept is a dimeric fusion protein made up of the human IgG1 Fc domain and the TNFR2 p75 subunit. The average half-life of etanercept is 102 hours which is the shortest among TNFi. RA patients using etanercept therapy receive the subcutaneous injection of 50 mg every week. Infliximab is a chimeric mouse anti-human IgG1 monoclonal antibody. Infliximab reduces multiple proinflammatory cytokines including interleukin-6 (IL-6) and acute-phase protein including fibrinogen in RA patients. The half-life of infliximab is 7.7-9.5 days and RA patients using infliximab receive intravenous induction of 3mg/kg every 8 weeks. Similar to adalimumab, golimumab is a fully human antibody. The average half-life of adalimumab is about 2 weeks and RA patients receive the subcutaneous injection of adalimumab 40 mg every 2 weeks while the average half-life of golimumab in RA patients is about 12 days and RA patients receive an intravenous infusion of golimumab 2 mg/kg every 8 weeks. Certolizumab pegol only contains one Fab fragment to recognize TNF- α but extends the half-life by conjugating with polyethylene glycol (PEG). The average half-life of certolizumab pegol is about 14 days. RA patients using certolizumab pegol receive 200 mg subcutaneous injection every 2 weeks.

3. Discussion

Although evidence suggests that TNFR2 regulates T_{reg} cells, the clear mechanism requires more investigation. The absence of TNFR2 in mice without the CIA model has no effect on T_{reg} cells but decreases Foxp3 by the demethylation of CpG motifs of Foxp3 compared to WT mice. However, the TNFR2-deficient mice CIA model shows a decreased number of T_{reg} cells and more severity and arthritis compared to WT mice [23]. This suggests that TNFR2 may affect the suppressive function of T_{reg} cells on inflammation. Foxp3 expression, maintenance, and proliferation are all increased in cultured T_{reg} cells by TNFR2 signaling, which also identifies a subset of T_{reg} cells that undergo hypomethylation, which results in stable Foxp3 expression [27]. The canonical NF- κ B pathway significantly mediates the T_{reg} cells' survival by co-stimulating part of the members of the TNF superfamily or TNF receptor superfamily (TNFRSF). Progranulin is a ligand of TNFR2 which binds more specifically to TNFR2 than to TNF- α . Progranulin-deficient mice are more susceptible to CIA while the administration of recombinant progranulin reduces arthritis in progranulin-deficient mice [28]. 14-3-3 ϵ is isolated as a signaling molecule from the TNFR2 complexes under progranulin stimulation in macrophages. TNFR2/14-3-3 ϵ signaling is also discovered to upregulate anti-inflammatory T_{reg} cells. Compared with 14-3-3 $\epsilon^{fl/fl}$ mice in the mice CIA model, deletion of 14-3-3 ϵ decreased T_{reg} cells in the joints and spleen and prevented progranulin from upregulating T_{reg} cells [29].

The mechanism of the induction of osteoclast by TNF- α remains unclear. Evidence shows that TNF- α induces osteoclast differentiation independent of the RANKL/OPG signaling. Inhibitors of OPG and the Fab fragment of anti-RANK antibody are unable to reduce TNF- α -induced osteoclast, while antibodies against TNFR1/2 or depletion of TNFR1/2 manage to do so [30]. Osteoclast precursor cells prepared from RANK-knock-out mice induced obvious tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts under stimulation of TNF- α . These osteoclasts induced by TNF- α showed no bone-resorbing activity until IL-1 was added [31]. However, some data suggest that the induction of osteoclasts by TNF- α in vivo depends on the RANKL/OPG pathway. Researchers discovered TRAP-positive and cathepsin K-positive osteoclasts on the bone surfaces and cells that look like osteoclasts close to the administration location within the calvaria of the RANK^{-/-} mice challenged with TNF- α , but no signal of bone resorption was found. This suggests that in RANK-deficient mice, TNF- α may compensate for the formation of these osteoclasts nearby in an alternative way [32]. Another study shows that a pure population of RANKL-deprived osteoclast precursors cannot form osteoclasts treated with TNF- α alone. However, the immune-purified macrophages are enabled to initiate osteoclastogenesis by TNF- α after being exposed to a small amount of RANKL. Thus, TNF- α and RANKL may synergize in the induction of osteoclasts [33].

Treatment with anti-TNF- α monoclonal antibodies may induce anti-drug antibodies (AD) which reduces the concentration of TNFi in serum and may cause type I/III hypersensitivity responses. RA patients from Argentina receiving treatment with infliximab or adalimumab showed positive for ADAs while those who received etanercept did not [34]. For type I hypersensitivity, anti-infliximab antibodies of all isotypes—IgE, IgM, and non-isotype-specific—were found in patients receiving infliximab treatment [35]. Type III hypersensitivity includes the precipitation and deposit of large ADA/therapeutic protein complexes. Anti-adalimumab antibodies were detected in patients with the treatment of adalimumab. Venous and arterial thromboembolic events appeared more commonly in these patients [36].

In RA, TNF- α may regulate hypoxia-inducible factor 1- α (HIF-1 α). The combination of TNF- α and IL-17 activates HIF-1 α by the NF- κ B pathway and induces the migration and the invasion of synoviocytes by activating the CXC chemokine receptor 4 (CXCR4)/stromal cell-derived factor 1 [37]. B cell activating factor (BAFF) increases in patients suffering from autoimmune disease, especially in the synovium [38, 39]. TNF- α induces BAFF which further upregulates vascular endothelial growth factor in synovium through the expression of c-Fos [40]. The expression of BAFF upregulated by TNF- α in synoviocytes requires HIF-1 α as transfection of HIF-1 α -siRNA significantly downregulated the promoter activity of BAFF stimulated by TNF- α while the overexpression of HIF-1 α or the hypoxia condition enhances the expression of BAFF [39]. Collectively, the TNF- α -induced HIF-1 α signaling pathway may be another target to develop new clinical treatments for RA patients.

4. Conclusion

TNF- α is an essential regulator of the cytokine signaling pathways in the pathogenesis of RA. TNF- α forms different complexes with its receptors and other molecules to induce the pro-survival, apoptosis, or necrosis of the cells. In RA, TNF- α produced by different cells carries out multiple functions. The regulation of RA by TNF- α results from the methylation and acetylation of relevant genes and differentiation of osteoclasts. TNFR1 promotes RA through the NF- κ B signaling pathway while TNFR2 downregulates RA by inducing T_{reg} cells. The clinical TNFi is administrated for RA patients routinely. The precise mechanism of the regulation of T_{reg} cells by TNFR2 and the induction of osteoclasts by TNF- α needs further research and may provide new targets for the treatment of RA.

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