

## TGF beta in the rheumatoid arthritis research

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### Mini-Review

**Abstract:** Rheumatoid arthritis (RA) is a chronic inflammatory disease of joints primarily, which is characterized by immune cell infiltration and hyperplastic growth of resident cells and blood vessels in synovial tissues. Despite that biomarker research in RA has progressed greatly, the pathogenesis is still not well defined. Transforming growth factor TGF beta is a homodimeric cytokine with multiple functions in embryonic development, immune responses, inflammation and repair. The involvement of TGF beta in RA has been under investigation for a long period in various contexts. The structure and activation of TGF $\beta$ 1, TGF $\beta$ 2 and TGF $\beta$ 3 have been reviewed and compared in this review. Its profibrotic and immunosuppressive function has been discussed in multiple diseases especially in terms of RA pathogenesis.

**Key words:** Rheumatoid Arthritis (RA); Transforming growth factor (TGF) beta; (TGF) beta receptor (TGF $\beta$ ); Osteoarthritis (OA); autoimmune disease.

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### Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic immune cell infiltration, hyperplastic growth of resident cells and blood vessels in synovial tissues and invasiveness of inflammatory components to adjacent tissues (1,2,3). Insufficient treatment of RA can result in joint dysfunction, chronic joint deformity, reduced lifespan, and even increased mortality rate (2,3). Despite that enormous effort has been put in RA biomarker research, the pathogenesis of RA is not totally known (3). TGF beta is a well-known cytokine that is important in RA biomarker research. It is universally expressed and belongs to the activins/bone morphogenetic (BMP) protein family (2,4,5). TGF beta exists in at least three homologues (TGF-beta-1, TGF-beta-2, and TGF-beta-3) and each form is involved in different diseases (1,4,6).

Multifunctional as it is, TGF beta is very critical for many biological processes including proliferation, wound healing, inflammation and repair, and immune responses (1-5). In terms of latent TGF beta structure and activation, TGF beta 1, 2 and 3 have similarities but also distinction especially in LAP domain as well as latency-integrin dependent activation switch (4). TGF-beta is also a fibroblast gene signature that can stimulate different human tissue fibroblasts via type II receptor for signaling (8, 9). There is evidence showing that TGF beta has a role in human RA, osteoarthritis (OA), collagen-induced arthritis and streptococcal cell wall (SCW) induced rat arthritis (1,6-9). And the mechanism underneath still needs further investigation.

### Pathogenesis of RA

RA is a very heterogeneous disease, which can be caused by multiple environmental and genetic factors. Genome wide analyses implemented suggested that im-

mune regulatory factors underlie the disease (1-5, 10). Several genes were reported to be associated with higher risk of RA including human leukocyte antigen (HLA)-DRB1, HLA-DRB1 and PTPN22 genetic interaction, etc. Synovial immunological processes and inflammation also plays an important role in RA pathogenesis. In addition to the genetics that contributes to RA, the presence of autoantibodies is another important factor, which places adaptive immunity at the center of early pathogenesis (10). Despite that Type 1 helper T cells are conventionally considered to be key in RA etiology, type 17 helper T cells (Th17) are also potential cell type that is critical in RA pathogenesis. Th17 cells produce interleukin-17A, 17F, 21, and 22 and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ). TNF- $\alpha$  is a well-established cytokine that causes RA etiology through multiple biological processes including cytokine and chemokine expression and activation, endothelial-cell adhesion molecules expression, synovial fibroblasts protection, angiogenesis promotion, regulatory T cells suppression, and pain induction. Macrophage-derived and dendritic-cell-derived transforming growth factor  $\beta$  and interleukin-1 $\beta$ , 6, 21, and 23 can facilitate Th17 differentiation, and also suppress regulatory T cell differentiation. This in turn disrupts T-cell homeostasis causing inflammation. Humoral adaptive immunity is another contributor to rheumatoid arthritis besides cytokines and associated pathways. Synovial B cells and anti-CD20 for B cell depletion indicate the critical role of humoral adaptive immunity for RA etiology. In summary, innate immunity, adaptive immunity, relative cytokine production and pathways associated all play important roles in RA pathogenesis (1-6, 10).

### TGF beta isoforms, structure and activation

In mammals, the 33 genes of the transforming growth factor beta (TGF- $\beta$ ) family each encode a polypeptide

constituting a secretion signal peptide, a pro-peptide domain, and a growth factor (GF) domain (9). The peptide structures of the TGF- $\beta$  isoforms are very similar with a 70-80% homology. TGF- $\beta$ 1 contains 390 amino acids and TGF- $\beta$ 2 and TGF- $\beta$ 3 each contain 412 amino acids. The signal peptide domain is at the N-terminus and contains 20–30 amino acids that are crucial for secretion of the cytokine from the cell. The pro-domain is called latency associated peptide (LAP), which are required for the proper folding and dimerization of growth-factor domain. The C-terminal region becomes the mature TGF- $\beta$  growth factor domain, which is released from pro-region by proteolytic cleavage (4,9-12).

Taekjip Ha et al has reported the activation process of TGF- $\beta$ 1, as a “caged” protein activated by integrin  $\alpha_v\beta_6$ . Ha et al proposed a model of force-induced activation of TGF- $\beta$ 1: In its latent form, TGF- $\beta$ 1 is surrounded by the “cage” pro-domain. Caged TGF- $\beta$ 1 is embedded in the extracellular matrix associated with an adaptor protein, both of which surround cells. Under activation conditions such as force and PH change, the cage opens and releases the TGF- $\beta$ 1. The growth factor can then bind TGF- $\beta$  receptor protein and induces downstream signaling (12). In summary, tensile force by integrins across the latent TGF binding proteins (LTBPs–prodomain–TGF $\beta$  – complex), is proposed in working models to change the conformation of the prodomain and to free TGF- $\beta$  for receptor binding and downstream signaling pathway (4,9, 12).

Notably, unlike TGF- $\beta$ 1 and TGF- $\beta$ 3, the pro-domain of TGF- $\beta$ 2 lacks a recognizable integrin binding motif such as RGD domain, which is recognized by  $\alpha_v\beta_6$  integrin (4,9,12). Therefore, the mechanism of TGF- $\beta$ 2 release from the “cage” and activation is unknown.

### TGF beta-receptor binding and signaling

TGF- $\beta$  has been used in various culture systems to understand its effects in stimulating osteoarthritic chondrocytes, RA fibroblasts and OA fibroblasts, renal fibroblasts etc (7,8, 13-15). Bira et al reported that TGF- $\beta$  stimulates rheumatoid synovial fibroblasts via the type II receptor (8). There are three classes of cell surface TGF- $\beta$  binding proteins known as TGF- $\beta$  receptors (R) I, TGF- $\beta$  RII and TGF- $\beta$  RIII. Unlike TGF- $\beta$ RI and RII, which contains a kinase domain to transduce intracellular signals, type III receptor can not transduce intrinsic signals. Once released, TGF- $\beta$  modulates signals through type I and type II receptors complex, which is low-affinity (type I) receptor by the ligand-bound high affinity (type II) pair (8, 11, 16). Type II receptor binds bio-active TGF beta and type I receptor is subsequently recruited and phosphorylated to regulate TGF beta signaling, while TGF beta RIII can stabilize the interaction between TGF beta RII and TGF beta RI (8,16). Following that, type I receptor then recruits and phosphorylates a receptor regulated SMAD (R-SMAD). The R-SMAD then binds to a complex, which then enters the cell nucleus and acts as a transcription factor to regulate various genes, including those to activate the apoptosis related mitogen activated protein kinase 8 (MAPK8) pathway. There are also feedback inhibition mechanisms of the SMAD pathway.

As was motioned, Bira et al examined the expression of TGF- $\beta$  receptors in synovial fibroblasts of RA patients and demonstrated the significant response of synovial

fibroblasts to TGF- $\beta$  stimuli. They have shown that synovial fibroblasts of RA patients expressed increased level of TGF- $\beta$  type II receptor, which is associated with elevated connective tissue growth factor (CTGF), chemotaxis and proliferation of RA synovial fibroblasts. All the biological processes might be involved in RA pathogenesis (8). For the ligand-receptor interaction, the N-terminal of TGF- $\beta$  RII (seven residues) and the prehelix of TGF- $\beta$  RI (five residues) play critical roles(15, 16).

### TGF beta, an immunosuppressive cytokine, profibrotic or anti-fibrotic?

The dysfunction of immune system or the disruption of immune homeostasis is related to multiple disorders like fibrosis, ageing, inflammatory bowel disease, cancer, autoimmune diseases, atherosclerosis, hypertension, osteoporosis and inflammatory diseases (1,2, 17-23). The effect of TGF beta in immune system and inflammation is complex given its various functions in multiple diseases. It was observed in studies that genetic deficiency of TGF- $\beta$ 1, the most potent TGF- $\beta$  isoform, results in autoimmunity and inflammation in multi-organs (1). For lymphocyte function, TGF- $\beta$  induces expression of forkhead box protein 3 (FoxP3) in vivo and in vitro and regulates the differentiation and function of regulatory T cells (Treg) in mice. In addition, TGF- $\beta$  produced by regulatory T cells (Treg), can suppress immune responses in different cell types. TGF- $\beta$  can also induce T helper type 17 (Th17) differentiation in mice and human together with inflammatory cytokines. Furthermore, TGF- $\beta$  has a role in myeloid cell lineages. TGF- $\beta$  can suppress or change the activation, maturation, and differentiation of macrophages, dendritic cells (DCs), and neutrophils according to many studies (1,2, 5, 24). For instance, TGF beta combined with interleukin 4(IL4) and interleukin 13(IL13) can prime THP-1 macrophages and primary human macrophages to M2 macrophage. TGF- $\beta$  induces M2-like macrophage polarization through the transcription factor SNAIL up-regulation (24). Therefore, TGF- $\beta$  is well known as an immunosuppressive cytokine and overexpressed in tumors, playing an important role in inhibiting anti-tumor response (1, 2, 5, 24, 25).

TGF- $\beta$  is also a fibrotic agent that is involved in numerous fibrotic disorders such as diabetic nephropathy, Crohn's disease, rheumatoid arthritis, radiation-induced fibrosis, and myocarditis (1, 13). It has been identified as one of the growth factors that can elicit a fibrotic response in the gut after inflammatory injury (5). Despite that both the TGF- $\beta$ 1 and TGF- $\beta$ 3 isoforms are expressed by fibroblasts from normal and inflamed mucosa, those tissues showed decreased TGF- $\beta$ 3 expression and increased TGF- $\beta$ 2 and TGF- $\beta$ 1 expression. This is notable given that TGF- $\beta$ 1 and TGF- $\beta$ 2 isoforms have been specifically implied in pathogenic fibrosis while TGF- $\beta$ 3 has antifibrotic characteristics (5, 25). There is an observation that  $\alpha$ (I) procollagen and TGF- $\beta$ 1 mRNA levels induced by TGF- $\beta$ 1 were decreased when both TGF- $\beta$ 3 and TGF- $\beta$ 1 were added to dermal fibroblasts. This indicates that TGF- $\beta$ 3 has antifibrotic traits in renal cells (14). Thus, TGF $\beta$ 3 has a distinct role in comparison to TGF- $\beta$ 1 and TGF- $\beta$ 2 given the role in fibrosis.

Given the complex role of TGF- $\beta$  in immune suppression, inflammation, fibrosis and anti-fibrosis, the role of

TGF- $\beta$  in RA is under debate. More research is required to study the specific homologues of TGF- $\beta$  and their function in RA etiology.

### TGF beta in RA synovium, synovial tissue and residing cells

Based on the in-vitro cell culture experiments, the role of TGF- $\beta$  in human rheumatoid synovial cells has been under debate, making the etiology of RA controversial (1,2, 25). According to many microarray and RNAseq analysis, myeloid cells including monocytes, macrophages and lymphoid cells include T lymphocytes and natural killer (NK) cells all express TGF- $\beta$ . Macrophages and T lymphocytes particularly are important cell source for TGF- $\beta$ , which is easily detectable in the synovium of RA patients (1). This is in accordance with the observation that TGF $\beta$ 1 is higher in synovial tissue of RA patients who have more myeloid cells in the joints and higher in RA/OA synovial tissue than normal. In addition, several studies have shown that of TGF- $\beta$ 1 is present in the synovial tissue and synovium of RA patients, and that TGF- $\beta$  receptor type II (TGF- $\beta$ RII) was higher in RA synovial tissue than normal (5). Cell type wise, TGF- $\beta$ RII was higher in fibroblasts compared to OA fibroblasts and that TGF- $\beta$  pathway associated genes including TGF- $\beta$ 1, TGF- $\beta$ 3, THBS1, LTBP1/2, SARA, TGFBR1 were elevated in RA fibroblast compared to OA fibroblast (2, 8). THBS1 is known to release active TGF- $\beta$  from its latent form. LTBP1/2 are both components of the large latent TGF- $\beta$  complex binding TGF- $\beta$  to the extracellular matrix; and SARA was reported to recruit the TGF- $\beta$  signal-transducing smads to the membrane close to the receptor. The elevated expression of TGF- $\beta$  and TGF- $\beta$  pathway associated gene signature in RA fibroblasts is noteworthy given that a study showed that TGF- $\beta$  and TGF- $\beta$ RI in rheumatoid synovial fibroblasts correlates positively with clinical markers of disease activity, indicating a correlation between TGF- $\beta$  and inflammation (2, 25). In addition to the elevated expression of TGF- $\beta$  receptor in RA fibroblasts, TGF- $\beta$  RI and TGF- $\beta$  RI appears to be higher in lymphoid cells including T cells and NK cells, and also myeloid cells including monocytes, macrophages, dendritic cells (DCs). Again, the higher expression of TGF- $\beta$  and receptors in some of the myeloid/lymphoid cells as well as the higher expression in the synovial tissue RA patients compared to normal indicate a potential role of TGF- $\beta$  in inflammation and RA disease pathogenesis (1,2, 10, 25)

The role of TGF- $\beta$  in inflammation has been known for a long time but the effect of rheumatoid synovium TGF- $\beta$  in in vitro studies with cells is disparate, acting as an immune inducer or inhibitor depending on the different surrounding environment (1, 2, 11, 19). It was classically considered that RA was an autoimmune disease that is mediated by Th1 cells producing IFN- $\gamma$  in the absence of Th2 cytokines. TGF- $\beta$  behaves as a negative modulator by inhibiting T-bet and GATA-3 transcription factors in both of the differentiated cell types. So TGF- $\beta$  was considered as an immunosuppressive agent classically, which was discussed as above. However, the proinflammatory effect in RA was known for contributing to RA pathogenesis in synovial lesion (1, 2, 5, 8, 25). TGF- $\beta$  elicits the secretion of inflammatory cytokines such as TNF $\alpha$ ,  $\alpha$  $\delta$  IL-1, acting as a potent chemoattractant for neutrophils, activating the

expression of chemokines, inducing expression of VEGF, which is key in the development of angiogenesis in RA, and modulating apoptosis of synovial fibroblasts (2). Therefore, whether TGF- $\beta$  promotes rheumatoid inflammation remains unclear (8).

### TGF beta in RA animal models

As was discussed above, the role of TGF- $\beta$  in promoting rheumatoid inflammation is controversial and not clear. This can be seen in multiple animal models of RA as well (1-3, 5-8, 26).

TGF- $\beta$ 1 was reported to have beneficial function in arthritis and suppress acute and chronic arthritis in experimental animal models (2,5,6, 8, 26). For instance, injection of TGF- $\beta$ 1 protects from arthritis development in collagen-induced arthritis (CIA) and systematic administration of TGF- $\beta$ 1 suppressed streptococcal cell walls (SCW)-induced arthritis (5,6). Again, there was a strong up-regulation of TGF- $\beta$ 1/2 in the remission state of disease in CIA model, which potentially indicated the anti-inflammatory regulation of T-cells by TGF- $\beta$  in arthritis. Mutations of TGF- $\beta$ 1 give rise to serious inflammatory disorders and functional absence of TGF- $\beta$ 1 causes an accumulation of cells and pro-inflammatory cytokines (TNF $\alpha$ , IFN- $\gamma$ , IL-1 $\beta$ ) (2,5,8). Sancho et al showed that blockade of TGF- $\beta$  by the antibody 1D11 during the induction phase of CIA increases CIA severity in WT mice (26). Those studies all point to the direction of TGF- $\beta$  as a beneficial factor in arthritis.

On the other hand, TGF- $\beta$ 1 can also generate deleterious effect in joint inflammation depending on the cell types and conditions and thus in mouse models of RA as well (1,2, 8). The injection of TGF- $\beta$ 1 into the joint cavity of rats induced synovial erythema, swelling, and leukocyte infiltration (8). In another study, intra-articular administration of TGF- $\beta$ 1 induces inflammation and joint damage in healthy rats. In addition, anti-TGF- $\beta$ RI antibodies treatment showed reduced signs of arthritis and synovial pathology in collagen antibody-induced arthritis (CAIA) mice models (1, 2). In a similar study, mice treated with p17, a peptide that specifically blocks TGF- $\beta$  signalling, showed reduced severity of CIA in comparison to control mice (1). Those observations are consistent with the findings that active TGF- $\beta$  signalling is detectable in RA synovium, which indicates that TGF- $\beta$  is a contributor to the sustained inflammation that characterizes RA (1,2). The administration of a TGFBR1 inhibitor, which reduced the expression of vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-AA, TNF- $\alpha$ , and cellular proliferation, and subsequently the prevention of CIA. This again further highlights the role of TGF- $\beta$  in arthritis pathogenesis (5). In summary, the above observations stressed the important role of TGF- $\beta$  in inducing inflammation and arthritis.

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