Thymosin β4 in rheumatoid arthritis: Friend or foe (Review)

KYOUNG SOO KIM[1,2] and HYUNG-IN YANG[2,3]

[1]Department of Clinical Pharmacology and Therapeutics, College of Medicine, Kyung Hee University; [2]East-West Bone and Joint Disease Research Institute, and [3]Division of Rheumatology, Department of Internal Medicine, Kyung Hee University Hospital at Gangdong, Seoul 134-727, Republic of Korea

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Abstract. Rheumatoid arthritis (RA) has characteristic pannus tissues, which show tumor-like growth of the synovium through chronic joint inflammation. The synovium is highly penetrated by various immune cells, and the synovial lining becomes hyperplastic due to increased numbers of macrophage-like and fibroblast-like synoviocytes. Thus, a resultant hypoxic condition stimulates the expression of inflammation-related genes in various cells, in particular, vascular endothelial growth factor. Thymosin β4 (Tβ4), a 5-kDa protein, is known to play a significant role in various biological activities, such as actin sequestering, cell motility, migration, inflammation, and damage repair. Recent studies have provided evidence that Tβ4 may have a role in RA pathogenesis. The Tβ4 level has been shown to increase significantly in the joint fluid and serum of RA patients. However, whether Tβ4 stimulates or inhibits activation of RA immune responses remains to be determined. In the present study, we discuss the logical and clinical justifications for Tβ4 as a potential target for RA therapeutics.

Contents

Correspondence to: Dr Kyoung Soo Kim, East-West Bone and Joint Disease Research Institute, Kyung Hee University Hospital at Gangdong, 149 Sangil-dong, Gandong-gu, Seoul 134-727, Republic of Korea
E-mail: kimks@khu.ac.kr

Abbreviations: RA, rheumatoid arthritis; DMARDs, non-responders to disease-modifying antirheumatic drugs; Tβ4, thymosin β4; CIA, collagen-induced arthritis

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1. Introduction

Cytokines control an extensive range of inflammatory progressions that are associated with the pathogenesis of rheumatoid arthritis (RA). In arthritic joints, pro-inflammatory activities lead to the induction of autoimmunity, chronic inflammation, and, ultimately, joint damage (1). A better understanding of these pathogenic mechanisms has enabled the development of therapeutic agents to inhibit cytokine actions, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6, which have greatly contributed to the management of RA patients (2,3). Biological therapies have contributed innovative improvements to RA treatment (4). However, despite these improvements, 30-50% of RA patients who are non-responders to disease-modifying antirheumatic drugs (DMARDs) fail to respond to treatment with biologic agents (5). Furthermore, radiographic joint damage can proceed even when clinical disease reduction is achieved by biologic agents (6).

These results indicate that the inhibition of cytokine networks may not be satisfactory to suppress RA progression. Thus, there is a need for new therapeutic agents to target new molecules, which encouraged us to redirect our efforts to screen other therapeutic targets from joint synovial fluids of RA patients. Recently, we observed that the level of thymosin β4 (Tβ4), which has many biological roles, was significantly increased in the serum and joint fluids of RA patients (7,8). This review aims to discuss the current understanding of the potential role of Tβ4 in RA pathogenesis while addressing current knowledge regarding the association between Tβ4 and arthritic joints for RA management.

2. Rheumatoid arthritis

RA is an autoimmune inflammatory disease whose pathogenic mechanisms remain elusive. RA pathogenesis is attributed to a complex interaction between genetic and environmental factors (7). The class II major histocompatibility complex molecules HLA-DR1 and HLA-DR4 are regarded as major genetic risk factors for RA (8). Environmental components are also responsible for RA development. Cigarette smoking is the most common environmental trigger and predicts both the susceptibility and severity of disease. Other environmental triggers that predispose individuals to RA are exposure to infectious agents and an imbalance in steroid hormones (9). Recently, citrullination and anti-citrullinated peptide antibodies

have been scrutinized as triggers of immune responses to RA. The most plausible molecular mechanism by which citrullinated peptides/proteins are involved in RA pathogenesis is that the modified antigen resulting from cell damage or uncontrolled apoptosis may induce an immune response leading to autoantibodies against these peptide or the whole protein (10). Anti-citrullinated protein antibodies are used as diagnostic tests for RA as frequently as is rheumatoid factor. The two diagnostic markers have approximately equal sensitivity and specificity for RA (11).

Regardless of the exact trigger, during RA pathogenesis, the combination of synovial proliferation, angiogenesis, and immune cell infiltration transforms the normal synovium into 'pannus' tissue, which shows tumor-like growth and invasiveness. The deregulation of highly formed microvasculature cannot provide enough oxygen to the synovium, which forms a hypoxic microenvironment due to the increased metabolism of the expanding synovial pannus. This results in abnormal cellular metabolism and mitochondrial dysfunction, which, in turn, actively induce inflammation through increased production of reactive oxygen species (ROS) (12). Various factors are involved over the course of RA, of which oxidative stress plays an important role in RA pathogenesis (13), particularly ROS and reactive nitrogen species (RNS). Serum levels of individual ROS and RNS in RA patients were significantly high compared with healthy subjects.

Treatment with ascorbic acid as an antioxidant significantly reduced all ROS and RNS levels in RA patients. Additionally, most reactive species had a strong positive correlation with clinical and biochemical RA markers, which indirectly confirmed a role of oxidative and nitrative stress in RA pathogenesis (14). Furthermore, pro-inflammatory cytokines, such as TNF-α, IL-1, IL-6, and IL-17, play a more important role in inducing joint inflammation and bone and cartilage destruction via the activation of macrophages, fibroblast-like synoviocytes, helper-T cells, and osteoclasts (15). Thus, biologics that target cytokines may offer substantial improvements to current RA treatment. However, some patients do not respond to biologics or lose their crucial response. Thus, there is a need for new therapeutic agents against RA. Inflammatory cytokines and cell surface molecules interact with cell-surface receptors to activate various cell-signaling pathways following phosphorylation of kinase proteins. Among these kinases, the non-receptor tyrosine kinase family Janus kinase (JAK) plays a critical role in RA pathogenesis. Several JAK inhibitors have been developed as new therapies for RA patients. These inhibitors are effective in patients who do not respond to biological or synthetic DMARDs (16). However, these agents have adverse effects, such as infection and hyperlipidemia, which are largely related to their mode of action (17). Thus, new therapeutics should be developed for patients who are resistant to existing agents or who experience side-effects from these agents.

3. Thymosin β4

Research into Tβ4 can be traced back to the early 1980s. Thymosin, which was known to increase excretion of luteinizing hormone-releasing factor (18), was initially isolated from calf thymus and was also known to be ubiquitously present in most rat and mouse tissues. Tβ4 concentration in the spleen was higher than in other tissue types including brain, kidney, liver, and testis (19). High concentrations were also found in peritoneal macrophages (20). The highest Tβ4 content, as well as the highest biosynthesis rate, was observed in Epstein-Barr virus-transformed human B-cell lines across 28 different cell lines. The levels observed in these cells were estimated at 1 picogram (pg) of Tβ4 per cell, which is three-fold higher than that in rat peritoneal macrophages (21).

The physiological role of Tβ4 was first thought to be an inhibitor of actin polymerization *in vitro* (22). When synthetic Tβ4 was microinjected into epithelial cells and fibroblasts, the stress fibers in cells were reduced, and depolymerization of actin filaments was induced in a dose-dependent manner. This suggested that Tβ4 was a potent regulator of actin assembly. Thereafter, the mechanism by which Tβ4 inhibits polymerization of actin filaments was shown to induce a conformational change in actin monomers. Its binding induces spatial rearrangements within the small domain (subdomains 1 and 2) of actin monomers in solution (23). Furthermore, Tβ4 is, not only located in cytoplasm, but is also translocated into the cell nucleus by an active transport mechanism, suggesting that this peptide may also act as a G-actin sequestering peptide in the nucleus (24). Other physiological roles were characterized by promoting angiogenesis, cell migration, and proliferation, which are essential steps in the repair progression following injury (25,26). Thus, Tβ4 was treated as a potential therapeutic agent for wound healing (27,28). Additionally, due to its physiological role in angiogenesis and cell migration, Tβ4 was thought to stimulate tumor metastasis and to be a potential molecular target for tumor therapy (29). In fact, Tβ4 expression was detected in high levels in certain tumor cell types, including osteosarcoma, colon adenocarcinoma, esophageal squamous cell carcinoma, kidney and urinary bladder transitional carcinoma, lung cancer, and liver cancer (30). Increased Tβ4 expression may contribute to cell survival and resistance to apoptosis (31-33). One of the mechanisms by which Tβ4 increases cell survival may be by increasing the expression of anti-oxidative enzymes, anti-inflammatory genes, and anti-apoptotic enzymes, thus preventing cell death (34-36).

4. Thymosin β4 and rheumatoid arthritis

Considering that Tβ4 promotes angiogenesis, cell migration, and cell survival, it can be hypothesized that Tβ4 probably plays an important role in promoting the formation of pannus, which show tumor-like growth and increased angiogenesis during pathogenesis of RA. However, this hypothesis was not promising for inflammatory diseases because of the anti-inflammatory effect of Tβ4. The involvement of Tβ4 in RA pathogenesis was not well investigated at the time that we began to examine Tβ4 levels in joint fluid and serum in RA patients. Previous findings have reported that Tβ4 was involved in inflammation, but most observed anti-inflammatory effects of Tβ4. For example, Tβ4 inhibited expression of inflammatory mediators in endotoxin-induced septic shock (37). It also appeared to have an anti-inflammatory effect in neonatal rats by inhibiting microglia activation through microRNA146a (38), and it inhibited the activation of NF-κB in TNF-α-stimulated cells (39). Thus, Tβ4 was regarded as an agent of anti-inflammatory activity (40), while

another report concluded that Tβ4 stimulates proinflammatory cytokine secretion in human pancreatic cancer cells (41). Therefore, due to its anti-inflammatory effect, the association of Tβ4 with inflammatory diseases has not attracted much attention, as demonstrated by the small number of reports currently available. Serum Tβ4 level was significantly increased in patients with inflammatory bowel disease (42).

The possible association of Tβ4 with RA was first reported in a study of the human plasma proteome in RA patients in 2009 (43). To the best of our knowledge, we were the first to use ELISAs to measure Tβ4 level in the serum and synovial joint fluid of RA and osteoarthritis (OA) patients. The levels were approximately 10-fold higher in the serum and synovial joint fluids of RA patients compared with healthy controls and OA patients (44-46). In particular, Tβ4 serum level was positively associated with RA or OA disease activity. Tβ4 level in the synovial joint fluid of RA patients was significantly associated with matrix-degrading enzyme levels, such as matrix metalloproteinase (MMP)-9 and MMP-13, angiogenesis-medicated protein, vascular endothelial growth factor, and inflammatory cytokines such as IL-6 and IL-8. However, it was not associated with MMP-1, MMP-2, MMP-7, adiponectin, or lactoferrin. By contrast, none of these molecules were associated with Tβ4 level in the synovial joint fluid of OA patients. Thus, it is suggested that Tβ4 plays an important role in bone degradation and inflammation in arthritic joints of RA but not OA patients (44). Consistent with the finding of an association between Tβ4 and MMP expression, there are reports that Tβ4 stimulates different types of MMP in various cells in a cell-specific manner during wound repair (47). Increased MMP activity is required for cell migration during wound repair (48).

Notably, a plausible role for Tβ4 in bone degradation by stimulating MMP expression would be inconsistent with a beneficial role in bone metabolism, which Tβ4 has been reported to play (49) by suppressing osteoclastic differentiation in RANKL-stimulated mouse bone-marrow-derived macrophages through the inhibition of osteoclast-specific gene expression, as well as p38, ERK, and JNK phosphorylation and NF-κB activation (50). Furthermore, Tβ4 promotes differentiation and mineralization of MC3T3-E1 cells, which are an osteoblast precursor derived from mouse calvaria (51). Tβ4 siRNA transfection suppressed osteoblastic differentiation by reducing calcium nodule formation, alkaline phosphatase activity, and mRNA expression of differentiation markers in human periodontal ligament cells, cementoblasts, and osteoblasts (52). Therefore, increases in Tβ4 level in serum and synovial joint fluid in patients with RA can be explained as one of the host defense systems that protect joint bone against activated osteoclastogenesis during inflammation of arthritic joints.

5. Conclusions and future studies

Although Tβ4 seems to play several roles in RA pathogenesis, its mechanism of action remains to be elucidated. Whether the increased Tβ4 level in serum and joint fluid in patients with RA acts through a pro-inflammatory or anti-inflammatory action remains to be determined. Furthermore, an increased Tβ4 expression is not thought to be involved in inhibiting or stimulating bone erosion in arthritic joints. To further elucidate the role of Tβ4 in RA pathogenesis, first, the Tβ4 expression level should be measured in a collagen-induced arthritis (CIA) mouse model. If Tβ4 is elevated in a CIA mouse model, further investigations should determine whether targeting with Tβ4-specific antibodies alleviates RA symptoms. Tβ4-transgenic or knockout mouse models may be useful to determine whether Tβ4 stimulates or inhibits bone erosion during RA pathogenesis. In conclusion, whether increased Tβ4 expression in serum and joint fluid in RA patients is of benefit or harmful remains to be elucidated. Thus, further studies are needed to develop a therapeutic agent against RA.

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