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主　論　文　要　旨

No.1

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主 論 文 題 名：
Elucidation of the therapeutic effect of HDAC-selective inhibitors on collagen-induced arthritis
コラーゲン誘導性関節炎における HDAC アイソザイム選択的阻害剤の治療効果の解明

（内容の要旨）

Rheumatoid arthritis (RA) is an autoimmune disease that affects approximately 1% of the global population. It is an age-related inflammatory disorder of the synovium, characterized by the proliferation of synovial cells, neovascularization, and infiltration of T cells, resulting in varying degrees of synovial inflammation and damage to articular cartilage structures. This can lead to joint deformity, loss of function, and a high rate of disability. In this study, I aimed to investigate the therapeutic effects of novel compounds in RA through two studies.

First, I conducted a screening of compounds that promote the differentiation of follicular regulatory T (Tfr) cells. Tfr cells are known to regulate the differentiation of Tfh and B cells in the germinal center (GC) of secondary lymphoid organs, assist in the selection of high-affinity antibodies, and maintain immune homeostasis by suppressing autoantibody production. Therefore, understanding the differentiation mechanism of Tfr cells and artificially increasing their numbers could provide a new approach for the prevention and treatment of immune diseases caused by autoantibody production. I screened for compounds with Tfr-cell differentiation-promoting effects from the intestinal microbiota metabolite library and compounds in the SCADs kits, and conducted *in vivo* studies.

In this study, I evaluated approximately 40 metabolites and found that cadaverine and putrescine had the most pronounced effects on differentiation in an *in vitro* assay. However, subsequent *in vivo* studies in mice showed that cadaverine and putrescine did not increase the number of Tfr cells. This may be due to the lower plasma concentration of polyamines in the mice. The SCADs kits are a collection of inhibitors for various small molecule compounds. The results suggest that HDAC inhibitors significantly promote Tfr-cell differentiation, consistent with previous findings in our laboratory. However, previous studies have shown that oral administration of HDAC inhibitors to mice does not significantly increase Tfr cell frequency, number, or Tfr/Tfh cell ratio in lymph nodes

compared to controls. These results suggest that HDAC inhibitors may improve autoimmune diseases through a pathway other than promoting Tfr-cell differentiation. Therefore, my next study will focus on identifying the targets and mechanisms by which HDAC inhibitors improve rheumatoid arthritis.

In this study, the mechanisms by which TTA03-107, a selective inhibitor of HDAC1, suppresses autoimmune arthritis were examined. Histone deacetylases (HDACs) regulate transcription by controlling reversible histone lysine acetylation, and pan-HDAC inhibitors (HDACis) have been shown to have therapeutic effects in rodent models of autoimmune diseases and in tissue samples from patients. However, their adverse effects, including fatigue, anorexia, diarrhea, vomiting, weight loss, and changes in serum biochemical markers, have limited their clinical use in the treatment of rheumatoid arthritis (RA). Therefore, HDAC isozyme-selective inhibitors may potentially have fewer adverse effects and be more effective in the treatment of chronic diseases like RA. Elevated transcript levels of HDAC1 have been identified in synovial fibroblasts and tissues of RA patients, suggesting that HDAC1 activity in T cells may be essential for RA pathogenesis and that selective inhibition of HDAC1 could be a promising therapeutic option for RA. Nevertheless, until recently, no HDAC1-selective inhibitors existed. In this study, the therapeutic effects of TTA03-107, were investigated in models of collagen-induced arthritis (CIA) and collagen antibody-induced arthritis (CAIA). Results showed that TTA03-107 inhibited macrophage cell differentiation and activity and attenuated the severity of arthritis in both CIA and CAIA models, with a higher rate of weight gain than the DMSO control group. Histological evaluations in the RA model showed that TTA03-107 treatment resulted in a significant reduction in infiltration of inflammatory cells and cartilage destruction in joint structures, with few signs of inflammation observed. TTA03-107 had no significant effect on CII-specific IgG. Analysis of serum and joint concentrations of TNF-α, IL-1β, and IL-17A showed that they were moderately reduced in the TTA03-107 group. These results suggest that TTA03-107 may alleviate the development of RA by suppressing the production of inflammatory cytokines rather than the CII-specific IgG immune response.

Therefore, I hypothesized that TTA03-107 could reduce the severity of arthritis by affecting osteoclast differentiation. *In vitro* experiments using bone marrow cells showed that TTA03-107 only partially inhibited osteoclastogenesis at high concentrations. Th17

cells have been linked to the development of osteoclastogenic potential in rheumatoid arthritis, leading me to hypothesize that TTA03-107 may inhibit cartilage and bone destruction in collagen-induced arthritis models by suppressing Th17-cell differentiation. In a Th17-cell differentiation culture system, TTA03-107 treatment led to a reduction in the frequency of RORγt^{+} Th17 cells, although no significant dose-response relationship was observed. Class I HDAC inhibitors have been shown to suppress the expression of inflammatory mediators in response to LPS stimulation in macrophages; although the involvement of HDAC1 is unknown, HDAC1 inhibition may also affect macrophage responsiveness. To further examine the potential anti-inflammatory effects of TTA03-107, I treated bone marrow-derived macrophages (BMDM) with the compound under M1 polarized conditions. My results showed that TTA03-107 treatment led to a significant increase in supernatant cytokine levels, as well as a decrease in the expression of M1 macrophage markers and an increase in M2 macrophage markers. These findings suggest that TTA03-107 may reduce the expression of proinflammatory cytokines secreted by M1 macrophages and promote the conversion of M1-type macrophages to anti-inflammatory M2-type macrophages.

In conclusion, to improve treatment options for patients with rheumatoid arthritis (RA), future research should investigate the similarities and differences of selective inhibitors targeting different histone deacetylase enzymes (HDACs). By understanding these mechanisms, we can identify selective agents with the most favorable safety and efficacy profiles, potentially leading to breakthrough therapies for RA and other autoimmune diseases.