News Release

Title

Soluble Siglec-9 suppresses arthritis in a collagen-induced mouse model and inhibits M1 activation of RAW264.7 macrophages

Key Points

○ Soluble Siglec-9 (sSiglec-9) reduced the incidence and severity of arthritis in a mouse RA model.

○sSiglec-9 also reduced M1 macrophage activation via NF-kB suppression in vitro.

○This new agent could contribute to RA treatment strategies via a new mechanism of action.

Summary

Clinical Associate Prof. Nobunori Takahashi (corresponding author), Takuya Matsumoto (first author) at Department of Orthopaedic Surgery and Rheumatology, Nagoya University Hospital (Director: Naoki Ishiguro , M.D., Ph.D.), and Akihito Yamamoto at Department of Oral and Maxillofacial Surgery / Protective Care for Masticatory Disorders, Nagoya University Graduate School of Medicine(Dean: Masahide Takahashi, M.D., Ph.D.), and Prof. Makoto Sawada at Research Institute of Environmental Medicine, Nagoya University, and Prof. Koichi Furukawa at College of Life and Health Sciences, Chubu University developed this study to assess the effects of soluble Siglec-9 (sSiglec-9) on joint inflammation and destruction in a murine collagen-induced arthritis (CIA) model and in monolayer cultures of murine macrophages (RAW264.7 cells and peritoneal macrophages[pMAC]).

sSiglec-9 significantly suppressed the clinical and histological incidence and severity of arthritis. Although sSiglec-9 reduced the expression of M1 markers in RAW264.7 cells and pMACs, it did not affect the expression of M2 markers and MMPs in FLS. NF-kB p65 phosphorylation was attenuated by sSiglec-9 in RAW264.7 cells.

In this study, we demonstrated the therapeutic effects of sSiglec-9 in a murine CIA model. The mechanism underlying these effects involves the suppression of M1 proinflammatory macrophages by inhibiting the NF-kB pathway. sSiglec-9 may provide a novel therapeutic option for rheumatoid arthritis patients refractory to currently available drugs.

Research Background

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by chronic

synovial joint inflammation, including synovial hyperplasia, infiltration of inflammatory cells, fibrin deposition, and joint destruction. Recent evidence suggests that the standard treatment strategies, including methotrexate-based conventional disease modifying anti-rheumatic drugs (DMARDs) and biological DMARDs, significantly suppress disease activity and joint destruction in RA patients. However, these strategies target only certain patient populations, and thus there is an unmet need for new anti-rheumatic drugs with novel mechanisms of action.

Sialic acid-binding immunoglobulin-type lectins (Siglecs) are type 1 transmembrane proteins that bind to sialic acid. Siglec-9 is a member of Siglecs which down-regulates both innate and acquired immune responses, and has immunoreceptor tyrosine-based inhibitory motifs (ITIM) in the cytosolic region.

Matsubara et al at Department of Oral and Maxillofacial Surgery / Protective Care for Masticatory Disorders, Nagoya University Graduate School of Medicine recently reported that the extracellular domain of Siglec-9 (soluble Siglec-9; sSiglec9) and MCP-1 (CCL2) induce functional recovery in a rat spinal cord injury model. They found that sSiglec-9 exerts anti-inflammatory effects at the spinal injury site by altering macrophage polarization from M1 (pro-inflammatory) to M2 (anti-inflammatory) dominant.

In this study, we used a mouse collagen-induced arthritis (CIA) model to investigate the therapeutic effects of sSiglec-9 in arthritis and subsequent joint destruction in vivo. We also conducted in vitro studies to evaluate the anti-inflammatory effects of sSiglec-9 in murine macrophages (RAW264.7 cells and peritoneal macrophages) and human fibroblast-like-synoviocytes (FLS).

Research Results

DBA/1J mice were immunized with type II collagen to initiate CIA. Mice were intravenously administered 100 µl of phosphate buffered saline (PBS) in the control CIA group or sSiglec-9 (5 or 50 ng/g body weight in a total volume of 100 µl) in the treatment groups weekly. Effects of sSiglec-9 were evaluated by physiologic arthritis score, histological analysis, serum tumor necrosis factor (TNF)-α concentration, and the proportion of forkhead box (Fox)-p3-positive regulatory T (Treg) cells.

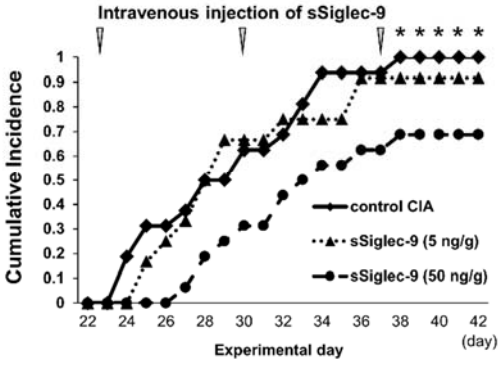
sSiglec-9 significantly suppressed the clinical and histological incidence and severity of arthritis (FIG1). The proportion of Foxp3-positive Treg cells significantly improved and serum TNFα concentration decreased in vivo.

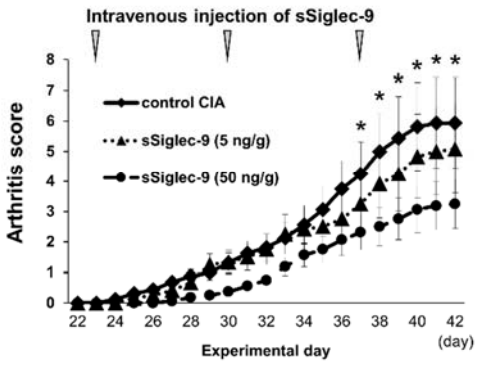
In vivo bio-fluorescent imaging was used to assess the distribution of sSiglec-9. Levels of M1 (TFNα, IL-6, and inducible nitric oxide synthase [iNOS]) macrophage markers and phosphorylation of intracellular signaling molecules were examined in macrophages,

and levels of matrix metalloproteinase (MMP)-1, MMP-3, and MMP-13 were examined in FLS.

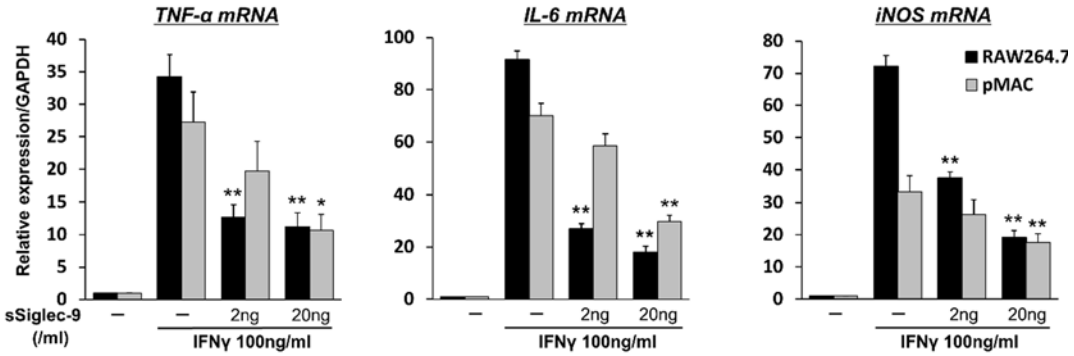
sSiglec-9 reduced the expression of M1 markers in macrophages (FIG2), however, it did not affect the expression of M2 markers and MMPs in FLS. NF-kB p65 phosphorylation was attenuated by sSiglec-9, and chemical blockade of the NF-kB pathway reduced M1 marker expression in RAW264.7 cells.

(FIG1)





(FIG2)



Publication

Matsumoto T, Takahashi N, Kojima T, Yoshioka Y, Ishikawa J, Furukawa K, Ono K, Sawada M, Ishiguro N, Yamamoto A. Soluble Siglec-9 suppresses arthritis in a collagen-induced mouse model and inhibits M1 activation of RAW264.7 macrophages. Arthritis Research & Therapy, June 7, 2016.

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http://www.med.nagoya-u.ac.jp/medical/dbps_data/_material_/nu_medical/_res/topix/2016/siglec9_20160624jp.pdf