



Original Full Length Article

Sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15) mediates periarticular bone loss, but not joint destruction, in murine antigen-induced arthritis



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ABSTRACT

Osteoclastogenesis requires immunoreceptor tyrosine-based activation motif signaling. Multiple immunoreceptors associated with immunoreceptor tyrosine-based activation motif adaptor proteins, including DNAX-activating protein 12 kDa (DAP12) and Fc receptor common γ (FcR γ), have been identified in osteoclast lineage cells, and some are involved in arthritis-induced bone destruction. Sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15) is an immunoreceptor that regulates osteoclast development and bone resorption in association with DAP12. Whether Siglec-15 is involved in arthritis-induced bone lesions, however, remains unknown. Here we used a murine antigen-induced arthritis model to examine the role of Siglec-15 in the development of bone lesions induced by joint inflammation. Arthritis was unilaterally induced in the knee joints of 8-week-old female wild-type (WT) and *Siglec-15*^{-/-} mice, and the contralateral knees were used as a control. The degree of joint inflammation, and cartilage and subchondral bone destruction in *Siglec-15*^{-/-} mice was comparable to that in WT mice, indicating that Siglec-15 is not involved in the development of arthritis and concomitant cartilage and subchondral bone destruction. On the other hand, the degree of periarticular bone loss in the proximal tibia of the arthritic knee was significantly lower in *Siglec-15*^{-/-} mice compared to WT mice. Although osteoclast formation in the metaphysis was enhanced in both WT and *Siglec-15*^{-/-} mice after arthritis induction, mature multinucleated osteoclast formation was impaired in *Siglec-15*^{-/-} mice, indicating impaired osteoclast bone resorptive function in the periarticular regions of the arthritic joint in *Siglec-15*^{-/-} mice. Confirming this result, *Siglec-15*^{-/-} primary unfractionated bone marrow cells harvested from arthritic femurs and tibiae failed to develop into mature multinuclear osteoclasts. Our findings suggest that Siglec-15 is a therapeutic target for periarticular bone loss, but not for joint destruction, in inflammatory arthritis, such as rheumatoid arthritis.

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1. Introduction

Three major forms of bone lesions are observed in rheumatoid arthritis (RA), including focal subchondral and marginal bone erosions,

periarticular osteoporosis around inflamed joints, and generalized osteoporosis [1–3]. Osteoclasts are thought to be primarily responsible for these bone lesions in RA, based on evidence that blockade of receptor activator of nuclear factor kappa-B ligand (RANKL) significantly reduces articular bone erosions and systemic bone loss in RA patients [4–6].

Osteoclast differentiation requires co-stimulatory signals mediated by immunoreceptor tyrosine-based activation motif (ITAM) harboring adaptors, such as DNAX-activating protein 12 kDa (DAP12) and Fc receptor common γ (FcR γ) [7,8]. Mice doubly deficient in DAP12 and FcR γ exhibit impaired osteoclast differentiation and severe osteopetrosis, indicating that the ITAM signals are essential for osteoclastogenesis [9,10]. Because both DAP12 and FcR γ have minimal extracellular domains, making them incapable of sensing signals outside the cell, immunoreceptors associated with DAP12 or FcR γ are thought to regulate ITAM signaling. Sialic acid-binding Ig-like lectin (Siglec)-15 is a DAP12-associated immunoreceptor that recognizes sialylated glycans and regulates osteoclast differentiation [11,12]. We previously

Abbreviations: AIA, antigen-induced arthritis; DAP12, DNAX-activating protein 12 kDa; FcR γ , Fc receptor common γ ; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; ITAM, immunoreceptor tyrosine-based activation motif; mBSA, methylated bovine serum albumin; micro-CT, micro-computed tomography; PBS, phosphate-buffered saline; PIR-A, paired immunoglobulin-like receptor A; qPCR, quantitative polymerase chain reaction; RA, rheumatoid arthritis; RANKL, receptor activator of nuclear factor kappa-B ligand; RT-PCR, reverse transcription-polymerase chain reaction; SD, standard deviation; Siglec-15, sialic acid-binding Ig-like lectin 15; TNF- α , tumor necrosis factor- α ; TRAP, tartrate-resistant acid phosphatase; UBMC, unfractionated bone marrow cells; WT, wild-type.

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demonstrated that Siglec-15 is involved in physiologic bone remodeling by modulating RANKL signaling [13].

Recent studies revealed that DAP12, FcR γ , and associated immunoreceptors are highly expressed in active RA synovial tissues compared to inactive RA or healthy synovial tissues, suggesting the involvement of ITAM signaling in the pathogenesis of RA [14,15]. Furthermore, several studies suggest that blockade of ITAM and associated molecules, such as FcR γ , paired immunoglobulin-like receptor A (PIR-A), and myeloid DAP12-associating lectin-1, reduces articular inflammation and cartilage and bone destruction in a murine arthritis model [16–18]. Therefore, whether Siglec-15 has a pivotal role in the pathogenesis and bone destruction of RA is of great interest. In the present study, we examined whether Siglec-15 is involved in destructive bone lesions induced by arthritis using a murine antigen-induced arthritis (AIA) model. AIA is a T cell-dependent arthritis model induced by antigen-specific T-cell activation.

2. Materials and methods

2.1. Mice and induction of AIA

The Ethics Review Committee for Animal Experimentation of Hokkaido University approved the experimental protocol. Mice were maintained under specific pathogen-free conditions. Both wild-type (WT) and *Siglec-15*^{-/-} mice were on a C57BL/6 background. Female mice were used at 8 weeks of age. AIA was induced as previously described. Briefly, mice were immunized at 8 weeks and 9 weeks of age by intradermal injection of 100 μ g methylated bovine serum albumin (mBSA; Sigma–Aldrich, St. Louis, MO) in 50 μ l phosphate-buffered saline (PBS), emulsified in 50 μ l complete Freund's adjuvant (Sigma–Aldrich) containing 4 mg/ml heat-killed *Mycobacterium tuberculosis* strain H37RA (Difco). Along with each immunization, 200 ng *Bordetella pertussis* toxin (List Biological Laboratories) was administered by intraperitoneal injection. Arthritis was induced at 11 weeks of age by injecting 100 μ g mBSA dissolved in 50 μ l PBS into the right knee joint, while 50 μ l PBS was injected into the left knee joint as a control [19]. The knee joint diameter was measured using a caliper from days 0 to 28. Joint swelling was expressed as the difference (in mm) between the right (mBSA) and left (PBS) knee joints. At 4 weeks after the intra-articular injections (day 28), mice were killed by an overdose of ketamine and xylazine, and knee joints were excised for histology and micro-computed tomography (micro-CT) analysis.

2.2. Micro-CT analysis

Both tibiae were scanned individually by micro-CT (R_mCT2; Rigaku, Tokyo, Japan) at a 10- μ m isotropic resolution and measured using a TRI/3D-BON (Ratoc System Engineering Co. Tokyo, Japan) in accordance with the guidelines described in Bouxsein et al. [20]. For tibiae, a 1000- μ m area of interest (100 slices) encompassing the region of the proximal metaphysis, starting from 300 μ m distal to the growth plate, was used to assess trabecular bone morphology.

2.3. Histology and histomorphometry

Both knee joints were fixed in paraformaldehyde, decalcified in EDTA, and embedded in paraffin. Sagittal knee joint sections were assessed by hematoxylin and eosin staining, and safranin O staining with fast green counterstain to evaluate joint inflammation and destruction, and stained with tartrate-resistant acid phosphatase (TRAP) with methyl green counterstain to observe osteoclasts. Arthritis was scored as follows: synovial hyperplasia (pannus formation), cellular exudate, and cartilage depletion/bone erosion were each scored from 0 (normal) to 3 (severe); synovial infiltrate was scored from 0 to 5 [21]. The osteoclast surface/articular surface, numbers of osteoclasts/bone surface, and osteoclast surface/bone surface at the secondary spongiosa

were determined according to Dempster et al. [22]. Primary spongiosa was defined as the area 250 μ m distal to the growth plate, and secondary spongiosa was defined as the area from 250 μ m to 1000 μ m distal to the growth plate.

2.4. Osteoclast cultures

Mouse primary unfractionated bone marrow cells (UBMC) were prepared from mouse femurs and tibiae. The osteoclastogenic potential of UBMC from WT and *Siglec15*^{-/-} mice with and without AIA was determined as previously described [23]. Briefly, mouse UBMC were seeded at 5×10^5 cells/well in 96-well plates and cultured in the presence of 50 μ g/mL ascorbic acid and 20 nM 1,25-dihydroxyvitamin D3. The medium was changed every other day throughout the study.

2.5. TRAP staining

Osteoclast generation was confirmed by TRAP staining. After aspirating the medium, cells were fixed with 4% formaldehyde containing acetone and citrate solution at room temperature for 1 min and stained for TRAP using a histochemical kit according to the manufacturer's (Sigma–Aldrich) instructions. Multinucleated osteoclasts were identified microscopically as TRAP-positive cells with at least three nuclei, and the number of cells in each well was quantified.

2.6. Real-time quantitative PCR of gene expression

The mRNA expression level within the joint and periarticular bone was measured using reverse transcription-polymerase chain reaction (RT-PCR). Joint and periarticular bone were harvested on days 3 and 7 after intra-articular injection. Total RNAs were isolated using a Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA). cDNA was synthesized from 1 μ g total RNA using reverse transcriptase and oligo-dT primers for quantitative PCR (qPCR) analyses. qPCR was performed as previously described. cDNA samples were analyzed for both the genes of interest and the reference gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [24]. The following primers were used: GAPDH: 5'-AGTCCG GTGTGAACGGATTG-3' (forward), 5'-GGGGTCGTTGATGGCAACA-3' (reverse), RANKL: 5'-AGCCGAGACTACGGCAAGTA-3' (forward), 5'-AAAGTACAGGAACAGAGCGATG-3' (reverse), interleukin 17: 5'-GGTC AACCTCAAAGCTTTAACTT-3' (forward), 5'-TTAAAAATGCAAGTAAGT TTG-3' (reverse). The amount of mRNA expressed was normalized to the GAPDH expression.

2.7. Statistical analysis

Data of two-group comparisons were analyzed using a two-tailed Student's t test. Simultaneous comparisons of more than two groups were performed using analysis of variance. A P value of less than 0.05 was considered statistically significant. Data are represented as mean \pm standard deviation (SD).

3. Results

3.1. Development of arthritis and joint destruction in *Siglec-15*^{-/-} mice was equal to that in WT mice

To investigate the contribution of Siglec-15 to inflammatory arthritis, we induced AIA in WT and *Siglec15*^{-/-} mice. All mice developed inflammatory arthritis after intra-articular injection of mBSA. Both WT and *Siglec15*^{-/-} mice exhibited a similar pattern of joint swelling over the 28-day time course, and there was no significant difference in joint swelling at any time point (Fig. 1A). We confirmed that there were no significant differences in the gene expression levels of interleukin 17 and RANKL between WT and *Siglec-15*^{-/-} on days 3 and 7 after arthritis induction (Fig. 1B).

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