Siglec15 Mediates Periarticular Bone Loss But Not Joint Destruction In Murine Antigen induced Arthritis

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Introduction: Rheumatoid arthritis (RA) is a systemic immune disorder characterized by polyarthritis. Three major forms of bone lesions can be observed in RA, including focal subchondral and marginal bone erosions, periarticular osteoporosis, and generalized osteoporosis. Osteoclasts are primarily responsible for these bone lesions in RA, supported by the evidence that blockade of RANKL significantly reduces articular bone erosions and systemic bone loss in patients with RA.

Sialic acid-binding immunoglobulin-like lectin 15 (Siglec-15) is an immunoreceptor that regulates osteoclast development and bone resorption in association with an ITAM harboring adaptor DNAX-activating protein 12kDa (DAP12). ITAM signaling, which is an essential pathway for osteoclast development, is activated by the immunoreceptors that associate with the ITAM adaptor proteins, DAP12 and Fc receptor common γ (FcRγ). MDL-1, a DAP12 associated immunoreceptor, was demonstrated to regulates synovial inflammation and bone erosion in mice model of RA; however, it remains unknown whether Siglec-15 is involved in development of bone lesions in RA.

In this study, we examined the role of Siglec15 in joint destruction and peri-articular bone loss in a mouse model of Antigen Induced Arthritis (AIA).

Methods: Mice and induction of AIA Both WT and Siglec-15/- mice were on a C57BL/6 background. Female mice were used at 8 wk of age. AIA was induced as previously described. Briefly, mice were immunized on days -21 and -14 by subcutaneous injection of 100 µg methylated bovine serum albumin (mBSA; Sigma-Aldrich) in 50 µl PBS and 50 µl Complete Freund’s Adjuvant (CFA; Sigma-Aldrich) containing 4 mg/ml heat-killed M. tuberculosis strain H37RA (Difco). Along with each immunization, 200 ng Bordetella pertussis toxin (List Biological Laboratories) was administered by intraperitoneal injection. Arthritis was induced on day 0 by injecting 100 µg mBSA dissolved in 50 µl PBS into the right knee joint, while 50 µl PBS was injected into the left knee joint as a control. 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 post intra-articular injections (day 28), mice were sacrificed and knee joints were excised for histology and micro-computed tomography analysis.

Micro-CT analysis Both tibiae were scanned individually by micro-computed tomography (CT, R_mCT2; Rigaku, Tokyo, Japan) at a 10-µm isotropic resolution. Both were measured using a TRI/3D-BON (Ratoc System Engineering Co. Tokyo, Japan) in accordance with the guidelines described in Bouxsein et al. For tibiae, a 1000-µm area of interest of (100 slices) encompassing the region of the proximal metaphysis, starting from 300 µm distal to the growth plate, was used to assess trabecular bone morphology. 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 (H&E)-staining to evaluate joint inflammation and destruction, and stained with TRAP with methyl green.
counterstain to observe osteoclasts. The gradation of 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. The numbers of osteoclasts/bone surface and osteoclast surface/bone surface at the secondary spongiosa were measured according to Parfitt et al. Primary spongiosa was defined as the area 250 µm distal to the growth plate and secondary spongiosa was defined as the area from 250 µm to 1000 µm distal to the growth plate.

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 ANOVA. A P value of less than 0.05 was considered statistically significant. The data are represented as mean ± SD.

Results: All mice developed an inflammatory arthritis in response to intra-articular injection of mBSA. Both WT and Siglec15-/- mice exhibited a similar pattern of joint swelling over a 28 day time course and there were no significant difference in right knee joint diameter at any time points. Histologically, WT and Siglec-15-/- mice comparably developed inflammatory arthritis characterized by extensive cellular infiltration, synovial hyperplasia, formation of a thick pannus, and bone erosions. Moreover, the degree of joint destruction assessed by 3D-reconstruction CT was similar between WT and Siglec-15-/- mice. Abundant osteoclasts are clearly visualized with TRAP staining as large red multinucleated cells at pannus and subchondral bone of knee joint in both WT and Siglec15-/- mice. These results indicate that Siglec-15 is not involved in joint inflammation and subchondral and marginal bone erosion in AIA. On the other hand, the degree of peri-articular bone loss seen in the proximal tibia after arthritis induction was significantly less in Siglec-15-/- mice. Although the osteoclast formation in the metaphysis area was enhanced in both WT and Siglec-15-/- mice after arthritis induction, the number of multinucleated osteoclasts was significantly lower in Siglec-15-/- mice compared to in WT mice. Importantly, osteoclasts in Siglec-15-/- mice failed to spread on bone surface, indicating impaired function of osteoclasts in Siglec-15-/- mice even under arthritis development.

Discussion: The experiments presented here show that Siglec-15 mediates periarticular bone loss but not joint destruction in inflammatory arthritis. The reason why osteoclast development is not impaired at pannus and subchondral bone of inflammatory joint might be explained by the compensatory mechanism for Siglec-15 deficiency. As we reported previously, Siglec-15 deficiency leads to impaired osteoclast development in the secondary spongiosa not in the primary spongiosa. We found that Siglec-15 deficiency can be compensated in the presence of type II collagen and bone matrix by stimulating osteoclast-associated receptor (OSCAR) /FcRγ signaling, which is alternative ITAM signaling pathway to DAP12. Therefore, inflammatory arthritis develops bone destruction independently of Siglec-15 signaling.

Our data and other previous studies of immunoreceptors suggest that multiple immunoreceptors, which associate with DAP12 or FcRγ, have different roles in terms of regulation of osteoclast development. Joyce et al. demonstrated that MDL-1, a DAP12 associated immunoreceptor, is a key regulator of synovial inflammation and bone erosion during autoimmune joint inflammation. Another study showed that bone erosion and osteoporosis but not inflammation, which were caused by aberrant TNF-α expression, were ameliorated in FcRγ deficient mice, in which the expression of PIR-A is impaired. On the other hand, Siglec-15 is essential for osteoclast development in secondary spongiosa under
inflammatory condition as well as physiological bone remodeling, but is not essential for joint destruction, which is controlled by other immunoreceptors.  

**Significance:** The findings of the present study indicate that Siglec-15 blockade is effective in reducing periarticular bone loss but not in joint destruction or inflammation in RA.

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