Resident non-classical monocytes are critically important for tissue destruction in arthritis

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Introduction

Bone erosions and systemic bone loss in rheumatoid arthritis patients results from an increased activity of osteoclast, which are derived from precursor cells of the myeloid lineage. Although there is much known about the mechanisms regulating the formation and activation of mature osteoclasts, the identity of an osteoclast precursor population in and its regulation by inflammatory cytokines during arthritis is poorly understood.

Methods

HTNFtg mice were clinically scored once per week for grip strength and swelling. In addition, blood was collected every week starting on week 4. Mice were sacrificed at week 10 - blood, spleen and bone marrow were collected for flow cytometry analysis. CCR2$^{-/-}$ mice were crossed into hTNFtg mice and histological analysis was performed. Different monocyte subsets were Facs-sorted and cultured in the presence of RANKL and MCSF to induce osteoclasts.

Results

Myeloid cells are elevated during arthritis

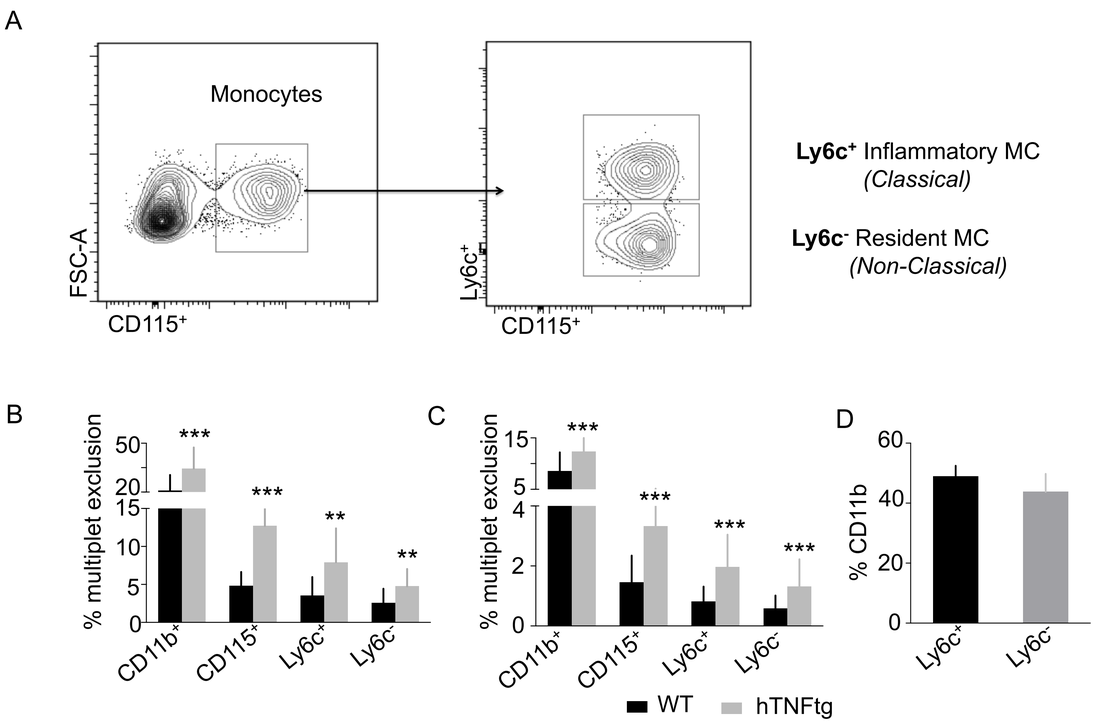

Figure 1. Myeloid cells are elevated in blood and spleen in hTNFtg arthritis.
A, Flow cytometry analysis of monocyte populations in blood, spleen and synovial tissue obtained from WT and hTNFtg mice. **B & C**, Bar graph summarizing frequencies of myeloid populations in blood (B) and spleen (C) and synovial tissue (D) of WT mice and hTNFtg mice. **p< 0.01; ***p< 0.001.

Resident MC arise before clinical onset of arthritis

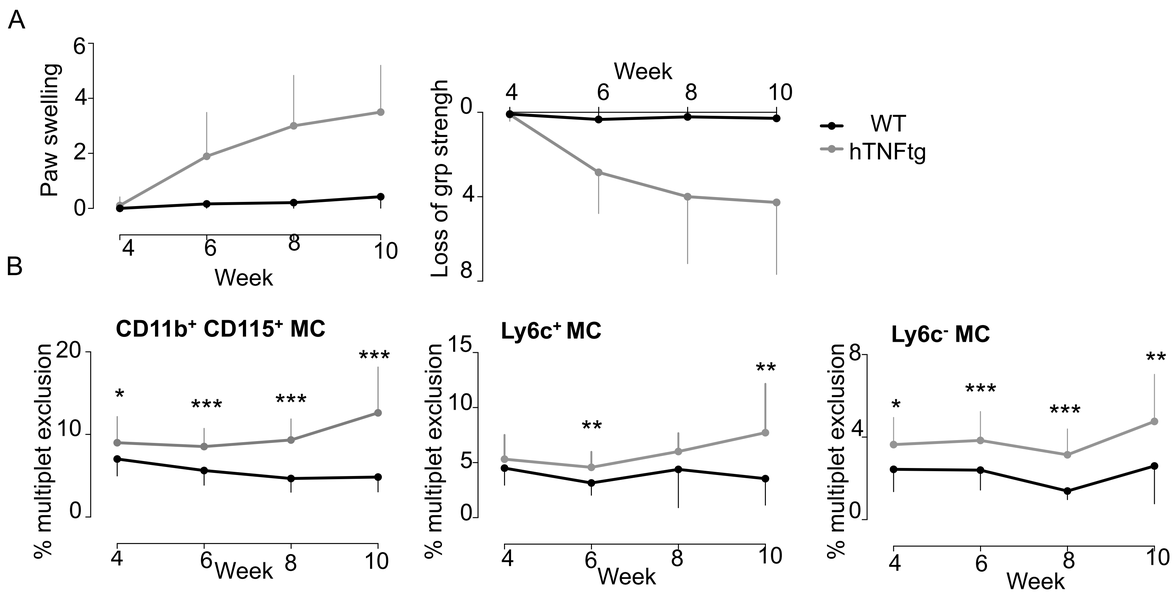

Figure 2. Development of arthritis is accompanied by accumulation of circulating mononuclear cells. A, Clinical assessment of paw swelling and grip strength in wild-type (WT) mice (n = 10) and hTNFtg mice (n = 9) . **B,** Characterization of Monocytes under Steady-State Conditions and during hTNF driven arthrtis.
Blood from WT mice and hTNFtg were analyzed using flow cytometry analysis. Times course showing appearance of Resident monocytes (Ly6c^{-} MC) in blood allready at preclinical stage of disease.

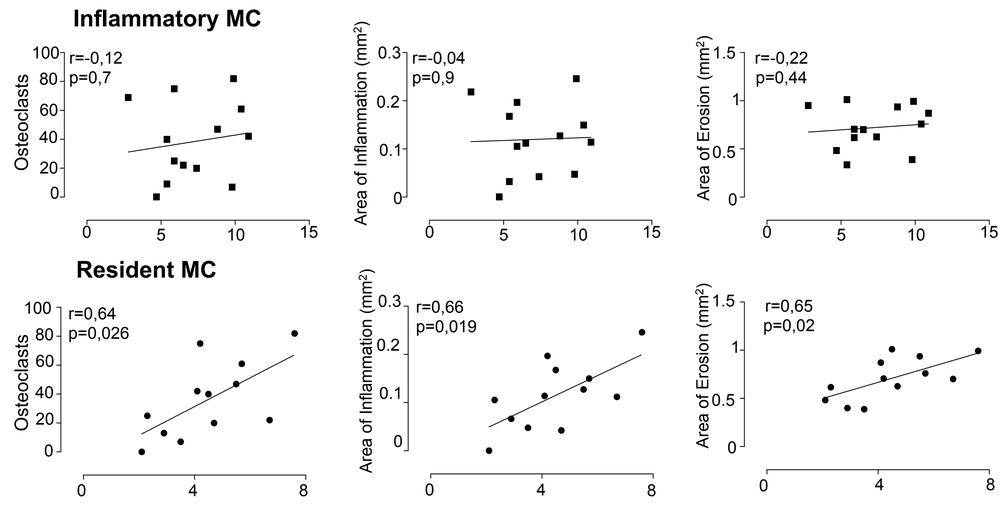

Figure 3. The number of circulating Resident MC, but not Inflammatory MC, correlates with the number of osteoclasts, the area of erosion and inflammation.

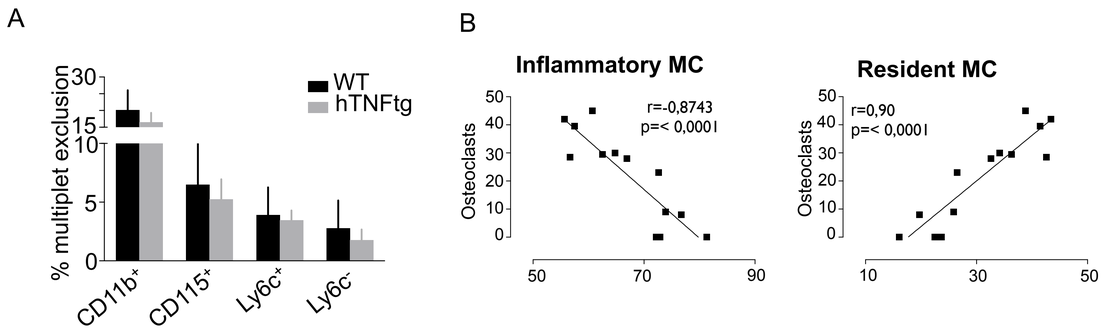

Figure 4. Analysis of monocyte populations in K/BxN serum transfer arthritis.
A, Flow cytometry analysis of monocyte populations in blood obtained from WT and mice in which K/BxN arthritis was induced. **B**, **C,** The number of Resident MC in blood correlate with the Area of inflammation, erosion and the number of osteoclasts.

Osteoclasts are formed from Resident MC

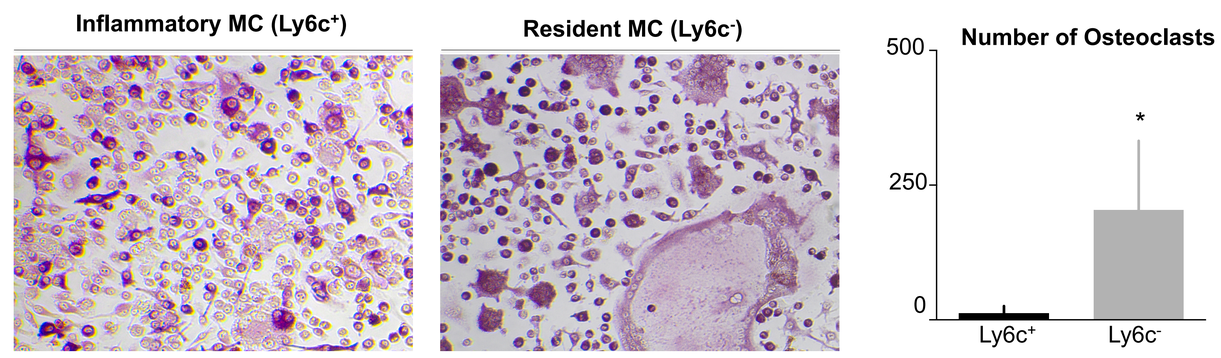

Figure 5. Identification of cell populations with osteoclastogenic potential from peripheral blood cells. Blood cells from WT mice and hTNFtg mice were stained with anti-CD11b and Ly6C antibodies and subjected to FACS analysis. Inflammatory MC and Resident MC were sorted and cultured with M-CSF and RANKL to generate osteoclasts.
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Mice lacking Inflammatory MC, display increased joint destruction

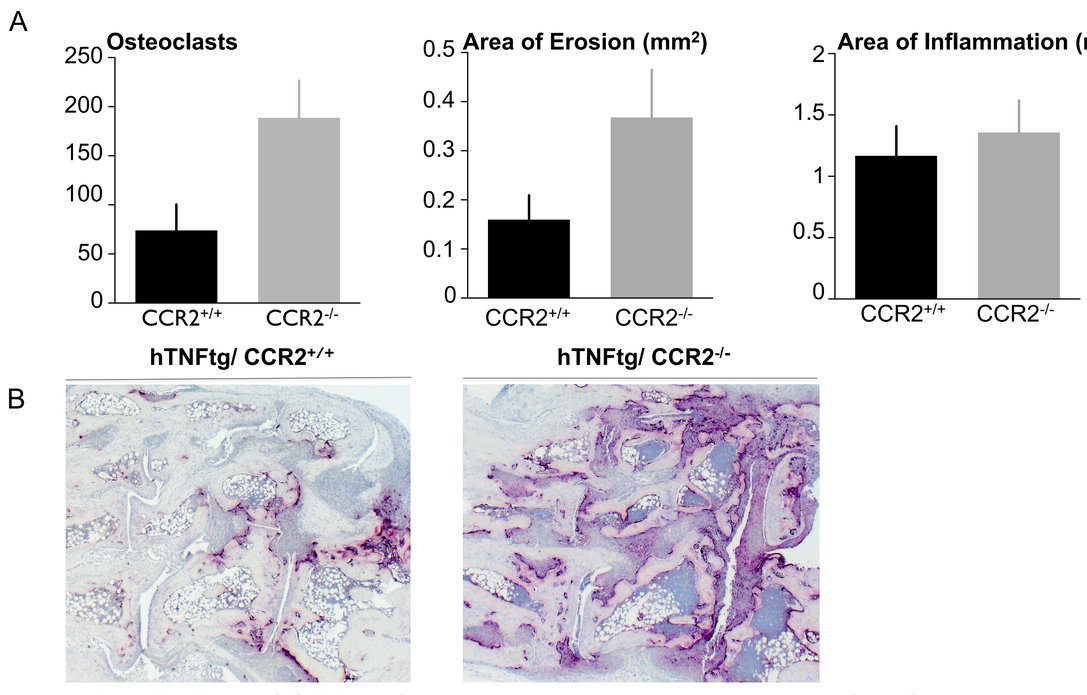

Figure 6. HTNFtg CCR2 deficient mice showed enhanced local bone destruction and osteoclast formation. CCR2$^{-/-}$ mice were crossed into hTNFtg mice and histological analysis was performed.

Conclusion

Resident non classical monocytes with osteoclastogenic potential are elevated during chronic inflammatory arthritis and the numbers in blood correlate with histological markers of joint destruction in models of inflammatory arthritis. Therefore these cells may provide a biomarker for erosive inflammatory arthritis and even a possible target for therapeutically intervention.