Dual-targeted Therapy Based On The Macrophage Niche In Rheumatoid Arthritis
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INTRODUCTION: Inflammatory infiltration and bone destruction are important pathological features of rheumatoid arthritis (RA), which originate from the disturbed niche of macrophages. Here, we identified a niche-disrupting process in RA: due to overactivation of complement, the barrier function of Fc/re4+ lining macrophages is disrupted and mediates inflammatory infiltration within the joint, and thereby activating excessive osteoclastogenesis and bone resorption. However, complement antagonists have poor biological applications due to super-physiologic dose requirements and inadequate effects on bone resorption.

METHODS: To investigate the pathogenic mechanisms and targets related to RA, we initially conducted an analysis of single-cell sequencing data from synovial CD45+CD11b+Ly6G+ monocyte-derived macrophages in the dataset GSE134420. This analysis aimed to explore the relationship between complement and the imprinting of macrophages in the joint microenvironment. To address excessive complement activation in RA, we developed a novel recombinant fusion protein, CRIg-CD59, and validated its anti-rheumatic activity in a rat model of antigen-induced arthritis (AIA). Following the identification of limitations such as dose dependency and limited effects on bone resorption of the protein, we further advanced the development of a dual-targeted therapeutic nano-platform based on a metal-organic framework (MOF). This platform enabled bone-targeted delivery of the complement inhibitor CRIg-CD59 and pH-responsive release. The surface mineralization of ZIF8@cRlg-CD59@HA@ZA targeted the acidic microenvironment of RA bone, while the sustained release of CRIg-CD59 could recognize and prevent the formation of membrane attack complexes (MAC) on the surface of healthy cells. Through Micro-CT, histological staining, and immunofluorescence analyses conducted on AIA rats treated with ZIF8@cRlg-CD59@HA@ZA, we verified the anti-RA activity of the dual-targeted nano-platform and its effects on Fc/re4+ lining macrophages and osteoclasts. Lastly, we focused on the characterization and validation of subpopulation features and proportions of joint macrophages using single-cell sequencing after treatment with ZIF8@cRlg-CD59@HA@ZA in RA.

RESULTS SECTION: GO and KEGG analysis revealed the central role of complement cascade in RA. Cell-specific GO analysis revealed significant enrichment tendencies of osteoclasts, further elucidating the relationship between complement and the imprinting of macrophages in the joint microenvironment: activation of osteoclasts and mediation of bone matrix remodeling. Recombinant protein CRIg-CD59 was constructed using seamless cloning and protein purification techniques. In vitro experiments confirmed the efficient expression of the protein and its anti-complement activity. The therapeutic results in rats with AIA model suggested that high-dose treatment with CRIg-CD59 significantly reduced IL-6 and TNF-α levels in RA but had limited alleviation of bone destruction. Subsequently, we prepared a dual-targeted nanoplatform, ZIF8@cRlg-CD59@HA@ZA, and validated its effective pH-dependent release, complement inhibition activity, bone targeting ability, and RA therapeutic activity through in vitro and in vivo experiments. Animal studies and single-cell sequencing results confirmed that the dual-targeted therapy could effectively suppress complement and osteoclast activation, while reducing M1 macrophages and increasing M2 resident macrophages, thereby improving the macrophage niche for the treatment of RA.

DISCUSSION: Various strategies have been tested in animal models using complement inhibitors, such as soluble CR1 (which inhibits complement activation at the C3 level), recombinant C3a and C5a receptor antagonists, and CD59 (which inhibits MAC formation), with promising results. Preclinical animal models of RA, selective administration of anti-C5 antibodies demonstrated therapeutic potential in preventing or treating arthritis. However, the C5aR inhibitor PMX53 and the anti-IL-6 receptor monoclonal antibody tocilizumab have both been found to be ineffective in treating RA. These observations indicate that additional investigation is required to identify complement as a potential therapeutic target for the treatment of RA. Our study highlights the limitations of single drug components in addressing complex pathological mechanisms of diseases, and suggests that nanoplatforms may not only provide efficient carriers for drug delivery but also offer comprehensive treatment of disease pathology through rational design to compensate for functional deficiencies of drugs. This provides a direction for future research and design of RA treatment strategies.

SIGNIFICANCE/CLINICAL RELEVANCE: This combination therapy is expected to treat RA by reversing the core pathological process, circumventing the pitfalls of traditional therapy.