Developing novel complement inhibitors targeting synovial lining macrophages

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INTRODUCTION: Rheumatoid arthritis (RA) is a chronic inflammatory disease affecting the facet joints. $CX3CR1_+$ synovial resident macrophages form a physical barrier to resist inflammation and stabilize joints. Imbalance in macrophage niches drives RA progression, involving recruitment of circulating macrophages, barrier disruption, and osteoclast activation leading to bone damage. Targeting synovial macrophage niches could provide novel therapies. Overactivation of the complement system, especially the alternative pathway, contributes to RA by forming membrane attack complexes (MACs) and causing tissue damage. Elevated complement products in RA synovial fluid can be reduced by antirheumatic drugs. Preclinical studies suggest potential for complement inhibitors (e.g., soluble CR1, C3a/C5a receptor antagonists, CD59) and anti-C5 antibodies, but clinical trials of PMX53 and tocilizumab failed. A fusion protein combining CRIg and CD59, targeting the alternative pathway and MAC formation, is being explored for RA treatment.

METHODS: We analyzed single-cell sequencing data from synovial $CD45^+CD11b^+Ly6G^-$ monocyte-derived macrophages to investigate the relationship between complement activity and macrophage imprinting in rheumatoid arthritis (RA). To explore changes in niche macrophages in pan-joint inflammation pathology, we constructed rat antigen-induced arthritis (AIA) and DMM-induced OA models, and stained for VSIg4 and CX3CR1. To combat excessive complement activation, we developed a novel recombinant fusion protein, CRIg-CD59, using seamless cloning and recombinant protein purification techniques, and evaluated its efficacy in a rat AIA model.

RESULTS SECTION: The study utilized single-cell sequencing from the GSE134420 dataset to explore synovial macrophage changes in rheumatoid arthritis (RA). It identified significant gene expression differences, including VSIg4, C4b, ACP5, CTSK, ATP6V0d, and C-FOS, across 18 cell clusters in the K/BxN STA model, highlighting their involvement in complement and coagulation cascades, cell activation, and extracellular matrix processes crucial for osteoclast differentiation and bone loss. Complement activation, pivotal in RA pathology, was confirmed using AIA rat models and DMM-induced arthritis models, showing excessive deposition of the membrane attack complex (MAC) on cartilage and synovial cells, leading to tissue damage and inflammation. VSIg4+ macrophages, which inhibit the complement pathway by binding C3b, become depleted due to excessive complement activity, exacerbating joint damage and inflammation. This contrasts with osteoarthritis (OA), where mechanical wear primarily drives synovial proliferation, significantly reducing VSIg4+ cells. These results highlight the protective role of VSIg4+ macrophages in RA and their potential depletion as a key disease progression mechanism, suggesting new therapeutic targets. To mitigate complement activation in RA, a CRIg-CD59 fusion protein was developed, combining CRIg's IgV domain with CD59's structural domain. This protein specifically targets and inhibits complement activity, capturing intermediates of C5b8 and C5b9 in MAC formation and binding C3b/iC3b at activation sites. Produced and verified via SDS-PAGE and Western blot from an E. coli system, this fusion protein significantly reduced arthritis symptoms and inflammatory markers (TNF- α , IL-6) in AIA rat models, indicating its potential as an effective RA treatment strategy.

DISCUSSION: Various strategies using complement inhibitors have shown promise in animal models of RA. However, some inhibitors, such as the C5aR inhibitor PMX53 and the anti-IL-6 receptor antibody tocilizumab, have not demonstrated effectiveness in clinical settings. These findings highlight the necessity of further research into the complement system as a therapeutic target for RA. We identified a group of synovial lining macrophages that resist complement activation and confirmed their absence in synovial lesions. Based on this, we developed a novel recombinant protein complement inhibitor and validated its effectiveness in a rat RA model.

SIGNIFICANCE/CLIICAL RELEVANCE: This work has developed developed a novel RA therapy based on niche macrophages.

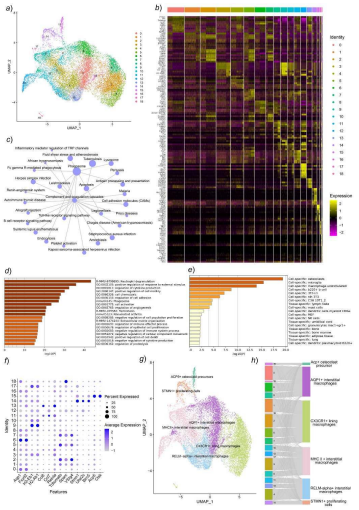

Figure 1. Identification of synovial niche macrophages. (a) UMAP analysis of synovial CD45+ CD11b+ Ly6G– monocyte-macrophages delineates the distribution of 18 subpopulations. (b) Heatmap analysis of significantly differentially expressed genes. (c)Pathway enrichment of DEGs revealed by KEGG; (d) Bar heatmap showing top annotated GO terms for DEGs for RA Day 5 and day 0. (e) Cell-specific enrichment of DEGs was determined in PaGenBase. (f) Dot plot of characteristic genes in macrophages. (g) UMAP plot of functional macrophage subgroups. (h) Sankey diagram showing the gene characteristic interactions and potential functional associations between functional macrophage subgroups.

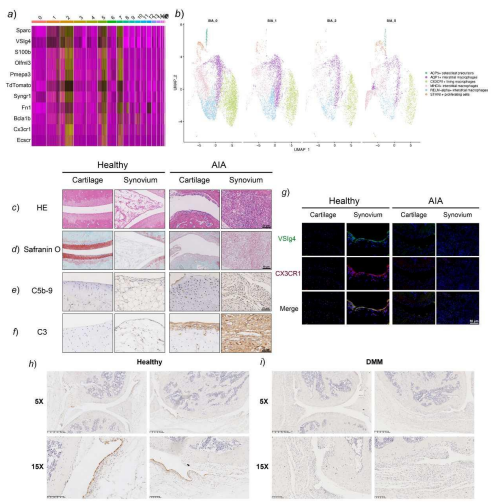

Figure 2. The absence of VSIG4+ niche macrophages in pathological synovium is associated with excessive complement activation. (a) Characteristic expression genes of the $VSIG4^+CX3CR1^+$ macrophage subgroup. (b) UMAP plots of changes in macrophage subgroups from Day 0 to Day 5. (c) HE and (d) Safranin O fast green staining, IHC for (e) C5b-9 and (f) C3, and (g) immunofluorescence staining for VSIg4 and CX3CR1 of SD rat joints 7 weeks after immunization with CFA in the footpad; Immunohistochemistry of VSIG4 in the joints of healthy mice (h) and mice undergoing knee medial meniscectomy (DMM) for 12 weeks (i).

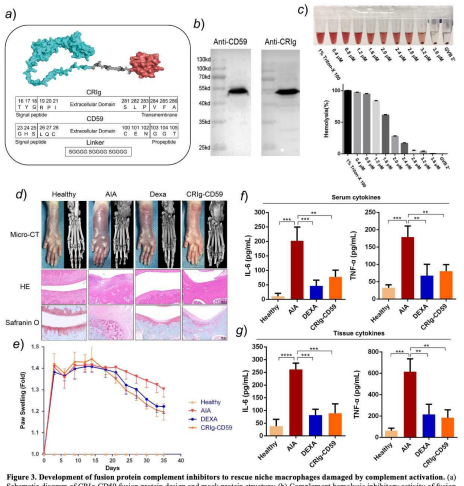

Figure 3. Development of fusion protein complement inhibitors to rescue niche macrophages damaged by complement activation. (a) Schematic diagram of CRIg-CD59 fusion protein design and mock protein structure; (b) Complement hemolysis inhibitory activity of fusion protein CRIg-CD59; (c) Micro-CT, HE staining and safranin fast green staining of joints in AIA model treated with CRIg-CD59 and dexamethasone for 21 days; (d) Statistics of paw swelling in AIA rats; Serum (e) and tissue (f) IL-6 and TNF-α protein levels in AIA rat model. Data are presented as mean ± SD (n = 3) and were statistically analyzed using a one-way ANOVA test by comparing to the control group. *p < 0.05, **p < 0.01 and ***p < 0.001. All results are representative data generated from at least three independent experiments. Data are presented as mean ± SD. The one-way ANOVA with the Tukey's multiple comparison test (h-i) was used for statistical analysis.