DOCTORAL THESIS

CRISPRa Engineered Elite Macrophages Enable Adoptive Cell Therapy for Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis (RA) is a chronic autoimmune disease marked by persistent synovial inflammation, leading to joint destruction, pain, and disability. Macrophages (MΦs) are central to RA pathogenesis, often towards the pro-inflammatory M1 phenotype, which contributes to the production of cytokines like TNF-α, IL-1β, and IL-6, exacerbating joint damage. Despite advancements in RA therapies, such as biologics targeting TNF-α, many patients remain refractory, highlighting the need for innovative therapeutic strategies. Macrophages exhibit plasticity, adopting different phenotypes in response to environmental cues. The M1 phenotype, induced by IFN-γ and microbial products, is associated with high pro-inflammatory cytokine production, whereas the M2 phenotype, induced by IL-4, IL-10, and IL-13, promotes anti-inflammatory and tissue repair functions. In RA, shifting from M1 to M2 holds therapeutic potential by reducing inflammation and promoting tissue regeneration. CRISPR-based transcriptional activation (CRISPRa) enables upregulation of specific genes, such as *IL-10*, in target cells without altering the genome. IL-10 is a potent anti-inflammatory cytokine that promotes the M2 phenotype. We hypothesized that CRISPRa-engineered macrophages (Elite MΦs) expressing IL-10 could serve as a novel therapy for RA. We employed CRISPRa to induce stable IL-10 expression in primary murine macrophages, delivered via lentiviral vectors targeting the *IL-10* promoter. Elite MΦs were characterized in vitro for polarization, cytokine production, and response to M1 and M2 inducers. Flow cytometry, ELISA, and migration assays were used to evaluate their properties. In vivo efficacy was tested in the collagen-induced arthritis (CIA) mouse

model, where Elite MΦs were administered after arthritis onset. Disease progression was monitored through clinical scoring, histopathological analysis, and serum cytokine measurement. Elite MΦs exhibited a stable M2 phenotype with high CD206, Arg1, and IL-10 expression, and low iNOS and TNF-α expression. They showed enhanced migration to inflamed tissues and resistance to M1 polarization, maintaining their phenotype even in the presence of IFN-γ and LPS. In the CIA model, Elite MΦs significantly reduced joint inflammation, synovial hyperplasia, and bone erosion, with decreased inflammatory cell infiltration and preserved joint architecture. Pro-inflammatory cytokines like TNF-α and IL-6 were reduced, while IL-10 levels were elevated in Elite MΦ-treated mice, confirming their anti-inflammatory effects. The efficacy of Elite MΦs in the RA model highlights the potential of CRISPRa-engineered macrophages as a novel cell-based therapy for autoimmune diseases. By stably expressing IL-10, these cells reprogrammed the inflammatory microenvironment, promoting tissue repair and reducing inflammation. Their enhanced homing to inflamed sites and resistance to M1-inducing signals suggest sustained therapeutic benefits. This study also emphasizes the versatility of CRISPRa in modulating gene expression, offering a safer and more precise approach compared to traditional gene editing. CRISPRa-engineered Elite MΦs represent a promising therapeutic strategy for RA, capable of effectively modulating the immune response and preventing joint destruction. This approach could be adapted for other autoimmune diseases where macrophage polarization is critical. Future studies will focus on optimizing delivery, persistence, and long-term safety in larger models, moving towards potential clinical trials.