Macrophage repolarization in a long-term culture synovitis model

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INTRODUCTION:
Macrophages exhibit distinct phenotypes and functions depending on the microenvironment. Macrophages are broadly categorized into two groups, pro-inflammatory (M1) macrophages and anti-inflammatory (M2) macrophages [1]. Naïve (M0) macrophages activated by IFN-γ and microbial products polarize to M1 macrophages and express high levels of the pro-inflammatory cytokines. Conversely, M0 macrophages activated by IL-4 or IL-10 show a low iNOS and IL-12 production but produce anti-inflammatory cytokines [2]. M2 macrophages are involved in tissue repair and facilitate angiogenesis. Abnormally elevated release of proinflammatory cytokines is a hallmark of osteoarthritis (OA) and synovitis; macrophages play a crucial role in producing inflammatory mediators. We hypothesize that macrophage repolarization occurs under long-term disease modeling of synovitis using a 3D coculture system. We used a microphysiological system and cocultured fibroblasts, endothelial cells, and macrophages to mimic the physiological synovial environment. We also accessed the key genes and downstream pathways that regulate macrophage activities using RNA sequencing.

METHODS:
Human bone marrow-derived MSCs-derived fibroblasts, GFP-positive HUVECs (Angio-Proteomie, MA, USA), and primary macrophages were loaded into a fibrin-GelMA hybrid gel system. Fibroblasts and HUVECs were seeded with M1 macrophages (M1 coculture group) or M2 macrophages (M2 coculture group) at concentrations of 5 million cells/mL, 5 million cells/mL, and 10 million cells/mL, respectively. Total RNA was extracted from the coculture scaffolds using Trizol, and RNA sequencing was performed by Novogene. A Human Cytokine Array / Chemokine Array 48-Plex (Eve Technologies, Calgary, Canada) was used to determine the level of cytokine secretion in culture supernatant at day 7, 14, 21, and 28. Triplicate samples were measured.

RESULTS:
Cytokine levels at different time points were compared with the level at day 7. In the M1 coculture group, inflammatory cytokines such as IL-6, IL-8, TNFα, IP-10, and IL-12p70 were reduced significantly after 28 days of incubation. However, cytokines involved in anti-inflammatory regulation (IL-4 and IL-13) increased at day 21 and day 28. In the M2 coculture group, the pro-inflammatory cytokines increased, and anti-inflammatory cytokines decreased. We further confirmed the inflammatory-related genes by RNA sequencing. In the M1 macrophage coculture group, canonical M1 functional markers such as *CXCL1*, *CXCL3*, *CXCL8*, and *IL6* decreased significantly after 4 weeks of incubation compared with week 1. *CCL13* and *CLEC4A*, which are recognized as canonical M2-associated genes, were enhanced at week 4. Besides, the level of *MMP28* which promotes M2 function was increased. Additionally, we observed higher induction of *CD200R1*, which tended to inhibit the expression of proinflammatory factors during the culture process. This suggests that M1 macrophages may polarize to an M2 phenotype. We also assessed the expression of key genes in the M2 macrophage coculture group, and the results showed that proinflammatory genes including *CXCL1*, *CXCL3*, *CXCL5*, and *IL6* were enriched at day 28. Changes in gene expression and the cytokines indicated that macrophages repolarized to alternative phenotypes.

DISCUSSION:
Macrophages acquire distinct phenotypes and biological functions during environmental stimulation [3]. Here, repolarization of macrophages was observed over a longer incubation period. A deep knowledge of molecular mechanisms of macrophage polarization facilitates potential clinical application and reprogramming. We investigated the downstream pathways using a 3D synovial model. Several signaling pathways coordinated with each other and directed macrophages phenotype, including the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway, Toll-like Receptor (TLR) signaling, and nuclear factor kappa B (NF-κB) pathway. These pathways would make potential therapeutic targets for interventional modulation.

SIGNIFICANCE/CLINICAL RELEVANCE:
We investigated the mechanism of macrophage polarization in a simulated synovial tissue. Repolarizing macrophages might be a promising strategy for the intervention of inflammatory diseases such as synovitis.

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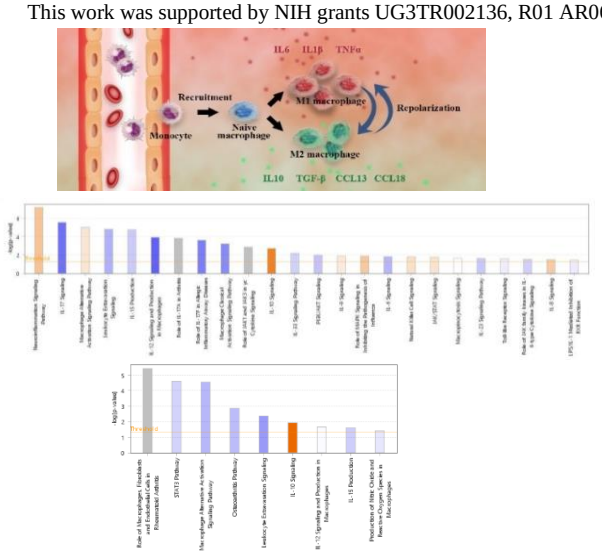

Figure 1 Top: Illustration of macrophage polarization and repolarization. Middle: Ingenuity pathway analysis of immune-related pathways in the M1 coculture group. Bottom: Ingenuity pathway analysis of immune-related pathways in the M2 coculture group.

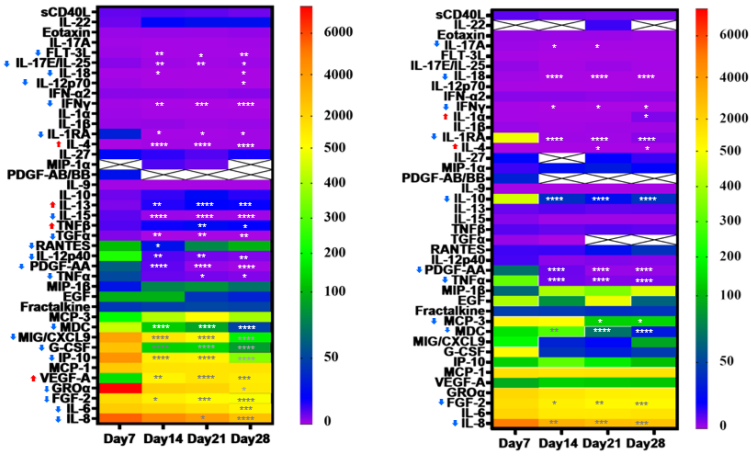

Figure 2 Inter-group comparison of cytokine levels with the cytokine concentration at day 7. Left: M1 coculture group. Right: M2 coculture group. * *P*<0.05, ** *P*<0.01, ***$P<0.001$, ****$P<0.0001$