Synthetic signaling to repurpose inflammation associated with arthropathy toward disease resolution

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INTRODUCTION: IL-1β and IL-6 are pro-inflammatory cytokines that play major roles in the pathology of arthropathies such as osteoarthritis (OA) and rheumatoid arthritis (RA).[1] Several IL-1β and IL-6 blockers in the form of antagonists or monoclonal antibodies have been clinically approved for use in treating arthropathies, though systemic administration of these drugs can have severe side effects including increased risk of serious infection. This motivates the need to develop avenues to antagonize cytokine signaling selectively and specifically in arthritic joints. The goal of our work is to engineer cells to sense and respond to elevated disease markers such as IL-1β and IL-6 and respond in a manner that resolves these chronic, deleterious arthritis inputs. Our prior work has demonstrated that cells engineered with synthetic Notch (synNotch)[2] receptors display spatially regulated responses to soluble inputs captured by a programmable biomaterial surface.[3] Here, we build off that work and developed methods that use IL-1β and IL-6 as inputs to activate engineered cells to respond via synthetic gene circuits to spatially constrain expression of pro-resolution transgenes. We hypothesize that combining a regenerative medicine approach with synthetic biology principles will allow for programmable responses to inflammatory cytokines that refine the treatment of arthritic joints.

METHODS: We used a synthetic receptor platform synNotch, which is based on the native Notch receptor, to recognize IL-1β and IL-6 and activate downstream gene circuits. Similar to Notch, synNotch requires anchored ligands to induce downstream signaling and has minimal response to monomeric, soluble ligands alone. As such, synNotch can be used to provide robust, localized transgene expression in areas with high levels of immobilized ligands. By leveraging synNotch in conjunction with ligand capturing biomaterials targeted to cytokines of interest, we can localize soluble IL-1β and IL-6 to desired regions and redirect cytokine signaling into transgene expression via synNotch signaling. To demonstrate cytokine dependent signaling, we developed IL-1β and IL-6 responsive mouse mesenchymal stem cell (mMSC) lines that express both a cytokine-specific synNotch receptor and a reporter mCherry and luciferase transgene payload. Both synNotch cell lines were tested using a functionalized cell culture plate coated with a monoclonal antibody that binds to an epitope of IL-1β or IL-6 that does not overlap with the epitope recognized by the cognate synNotch receptor. This allows for the immobilization of either IL-1β and IL-6 and subsequent activation of synNotch cells, which is measured via plate reader-based luminescence assays. Additionally, we measured our cells' ability to neutralize IL-1β and attenuate inflammation using a reporter cell line that produces mKate2 in response to NF-κB signaling, which is triggered in the presence of IL-1β and other inflammatory markers. mKate2 reporter expression was measured via flow cytometry. Lastly, we demonstrated that synNotch cell activation could be used to express the anti-inflammatory factors IL-4 and IL-10 in engineered mMSCs using a GFP responsive synNotch receptor. When co-cultured with macrophages, GFP-synNotch mMSCs equipped with the anti-inflammatory payload could polarize macrophages into a pro-resolution state in the presence of inflammatory cytokines. Results were quantified using qRT-PCR profiling various pro-resolution macrophage genes. Experiments were conducted in triplicates, and data were analyzed using ANOVA with α=0.05 and Tukey's post-hoc test.

RESULTS: Here, we demonstrate that mMSCs engineered with IL-1β and IL-6 responsive synNotch receptors can significantly activate reporter luciferase transgenes with cytokine concentrations as low as 1 ng/ml, with the most potent response at 10 ng/ml (Fig. A-B). Next, we interrogated whether our synNotch-monoclonal antibody system fully attenuated relevant inflammatory inputs when cells are not engineered to express therapeutic factors. After applying conditioned media from IL-1β synNotch mMSCs treated with IL-1β to NF-κB reporter mMSCs, we saw dose-dependent mKate2 expression, indicating that anti-IL-1β monoclonal antibodies and synNotch receptors were not sufficient to fully attenuate inflammation in the absence of engineered therapeutic transgene expression (not shown). These data suggest potential for our engineered cells to contribute toward inflammation resolution via re-routed cytokine signaling. Since inflammatory macrophages are commonly found at the site of joint injury, we hypothesize that polarizing macrophages toward an anti-inflammatory phenotype will reduce local inflammation that contributes to arthritis. Co-cultures of GFP responsive synNotch containing anti-inflammatory payloads with RAW264.7 macrophages led to upregulation of several pro-resolution genes such as *Mrc1, Cited2, Arg1* in macrophages (Fig. C).

DISCUSSION: We demonstrated that our approach of targeting the inflammatory cytokines IL-1β and IL-6 using synNotch receptors can lead to robust gene expression in the presence of our ligand immobilization surface. We have also shown that synNotch binding of inflammatory cytokines reduces inflammation, but alone is not sufficient for complete neutralization of inflammation. The addition of an anti-inflammatory transgene payload may further reduce inflammation in this context. Further applications of this technology include macrophage polarization via secretion of IL-4 and IL-10.

SIGNIFICANCE/CLINICAL RELEVANCE: Current IL-1β and IL-6 monoclonal antibody treatments have serious risks involved due to the immunosuppressive effects, and many patients develop resistances within several years of first administration. Our work aims to block inflammatory cytokines in a spatially dependent manner using customizable cell therapies in order to alleviate the problems with the current generation of drugs.

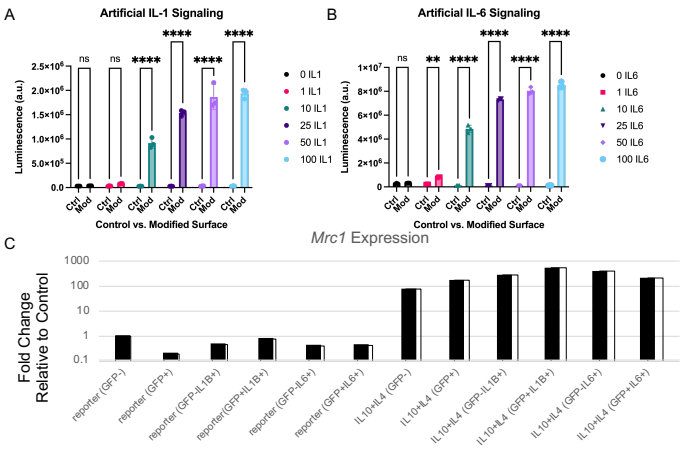

Fig. (A): Fold change luminescence activity of differentially treated IL-1β responsive synNotch lines cultured on control or IL-1β capturing surfaces. (B) Fold change luminescence activity of differentially treated IL-6 responsive synNotch lines cultured on control or IL-6 capturing surfaces. Cells are capable of artificially responding to as little as 1 ng/ml IL-6. (C) Representative qRT-PCR data showing enhanced *Mrc1* expression in RAW264.7 macrophages cocultured with synNotch mMSCs inducibly expressing IL-10 and IL-4. Treatment of cells with IL-β or IL-6 alone suppressed *Mrc1* expression. GFP-induced expression of IL-4 and IL-10 via synNotch resulted in enhanced expression of *Mrc1* despite IL-1β and IL-6 treatment of cells. These results suggest that future experiments with re-routed IL-1β or IL-6 signaling will enable polarization of macrophages based on synNotch transgene expression. Bars represent average n=3, **p<0.01; ****p<0.0001.

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