MSC Secretome as Potential Immunomodulatory and Regenerative Treatment Strategy for IVD Degeneration

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INTRODUCTION: Intervertebral disc (IVD) degeneration is triggered by trauma, altered loading, and genetic predisposition. Together, they induce a nucleus pulposus (NP) and annulus fibrosus (AF) cell-mediated synthesis of inflammatory cytokines. IL-1β has been identified as a critical mediator of disc degeneration and associated low back pain. (1) Blocking cytokine production at an early stage in the degenerative cascade might prevent disease progression and back pain. Besides their multineuage differentiation potential, mesenchymal stem cells (MSCs) release a broad spectrum of bioactive soluble factors, the so-called secretome. Importantly, MSCs adopt the profile of secreted proteins depending on their environment. (2) This allows them to take different roles tailored to the state of disease. The current project aimed to compare the anti-inflammatory and regenerative potential of different MSC secretome compositions. Human moderately degenerated NP-cells were exposed to IL-1β, mimicking an acute inflammatory stage. Human MSCs were primed with IL-1β, healthy, traumatic, and degenerative human IVD conditioned medium. Secretome was collected and applied on the inflamed degenerative NP-cell model. Treatment with unprimed MSC secretome, fresh serum-free medium, and dexamethasone+TGF-β3 was used as control.

METHODS: MSC secretome production: Degenerative and traumatic human IVD tissue was obtained with written consent from spine surgery patients. Healthy IVDs were harvested from organ donors after donor and familial consent. IVD tissue were incubated in culture medium for 48h to obtain IVD conditioned medium (CM). Human MSCs (n=12 donors) were isolated from bone marrow aspirates obtained with written consent from spine surgery patients. MSCs were primed with basal culture medium (BS), 10 ng/mL IL-1β (IL-1S), or IVD-CM from healthy (HS), traumatic (TS), and degenerative (DS) donors for 24h. Following, CM was replaced by fresh culture medium to generate MSC secretome for 24h. Secretome was pooled respectively.

Degenerative inflammatory NP-cell model (Fig. 1): Human NP cells were isolated from IVD tissue obtained with written consent from patients undergoing spine surgery (n=3, 33±2 years, Pfirrmann grade III), expanded (p3), and encapsulated in alginate beads at a density of 4x10^5 cells/mL. Following culture for 14 days, beads were exposed to 10 ng/mL IL-1β for 24h. On Day 15, the following treatment was applied: negative control (neg. ctrl. = no secretome applied), positive control (pos. ctrl. = treated with 10 ng/mL TGF-β3 and 10^-7 M dexamethasone), BS, IL-1S, HS, TS, and DS. After 24h of treatment = day 16, real-time quantitative PCR (qPCR) was performed, and media were replaced by a fresh culture medium to collect the factors released by the treated NP-cells. Culture medium was collected on day 17, and the concentration of ten human cytokines (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNFa, and IFNy) was quantified using a multiplex immunoassay. Statistical analysis: Normality was tested with the Shapiro-Wilk test. For parametric data, one-way ANOVA was performed. For non-parametric data, the Kruskal-Wallis test was performed; P < 0.05 was regarded as significant.

RESULTS: Multiplex immunoassay (Fig. 2): Compared to the neg. control, significantly lower concentrations of IL-2, IL-10, IL-12, TNFa, and IFNy were detected in the positive control. Treatment with IL-1S significantly reduced the release of IL-2, IL-4, IL-8, and IL-10. BS application led to significantly reduced release of IL-2, IL-4, IL-8, and IL-10. TS significantly reduced the release of IL-1, IL-2, IL-4, IL-8, and IL-10. Anabolic gene expression (Fig. 3): Expression of anabolic genes (ACAN, COL I, COL II) was normalized to neg. ctrl. on day 16 (n=3, mean ± SEM). Treatment by MSC secretome led to an upregulated expression of all measured genes. A significantly higher expression of Col II was observed following TS treatment compared to neg. ctrl.

DISCUSSION: Our data indicate that dexamethasone application reduces the amount of pro-inflammatory, and potentially pain triggering cytokines (IL-2, IL-12, TNFa, IFNy). Glucocorticoids (GCs) inhibit the expression of many inflammatory mediators and represent the current gold standard in the treatment of inflammatory pathologies. In our model, a single GC intradiscal injection was associated with a pain reduction in patients suffering from discopathy related back pain (3). In our model, GC application also reduced the release of cytokines associated with anti-inflammatory effects (IL-4, IL-10). Similarly, the application of IL-1S and BS also led to reduced IL-10, potentially attenuating an anti-inflammatory effect. In contrast, TS did not alter the secretion of IL-10 while also reducing IL-1β secretion, representing a key player in the IVD’s degenerative cascade. qPCR data indicates a higher expression of anabolic genes in the TS group compared to the neg. and pos. control. While HS did also induce upregulation of anabolic genes, it did not diminish the release of immunomodulatory cytokines. This supports the hypothesis that MSCs adopt their secretome depending on the exposed environment leading to a distinct biological effect.

SIGNIFICANCE/CLINICAL RELEVANCE: The difference among secretome-treated groups might result from stimulation of multiple pathways. In contrast to GC application, surface receptor activation might mimic an environmental change around the NP cell leading to a sustained biological effect. In summary, MSC secretome application might represent an alternative to GC application for anti-inflammatory and additionally regenerative purposes.


Figure 1 | Scheme representing design experiment
Human NP-cell alginate beads were cultured for 14 days. Day 14: Beads were exposed to 10 ng/mL IL-1β for 24 hours. Day 15: negative control (neg. ctrl.) treated with basal medium (BM), positive control (pos. ctrl.) treated with TGF-β3+dexamethasone. Secretome treated groups included application of BS, IL-1S, HS TS, and DS. Day 16: qPCR was performed after 24h of treatment and media were replaced to collect the factors released by the treated NP-cells on day 17.

Figure 2 | Quantification of immunomodulatory cytokines
Culture medium was collected on day 17, and ten human cytokines (IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNFa, and IFNy) were quantified. Data indicate difference to neg. ctrl. * = 0.05; ** = 0.01; *** = 0.001.

Figure 3 | Anabolic gene expression
Gene expression of anabolic genes (ACAN, COL I, COL II) normalized to neg. ctrl. on day 16. A significantly higher expression of Col II following treatment with degenerative MSC secretome compared to neg. ctrl. was observed (n=3, mean ± SEM). * = 0.05 vs. neg. ctrl.

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