Interactive Cytokine Regulation of Synoviocyte Hyaluronan and Proteoglycan 4 Secretion Rates
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Introduction: The synovial joint constitutes a complex, low-friction, low-wear load-bearing system that is normally in homeostasis. However, cartilage undergoes erosion in osteoarthritis, rheumatoid arthritis, and post-injury degeneration, in association with a decrease in boundary mode lubricating properties of the synovial fluid (SF). In the abnormal SFs, the concentration of lubricants, including hyaluronan (HA) and proteoglycan 4 (PRG4), are altered, although the underlying mechanisms of this are unknown. One source of HA and PRG4 in synovial joints is the fibroblast-like synoviocyte in the synovium. How SF cytokines regulate synoviocyte lubricant secretion remains to be established, in health, injury, and arthritis. In the latter situations, IL-1β, IL-17, IL-32, TGF-β1, and TNF-α are often present at elevated levels. The hypothesis of this study was that these cytokines regulate synoviocyte lubricant secretion individually and in combination, either in additive or synergistic fashion.

Materials and Methods: Cell Isolation. Synoviocytes from synovium of human patients undergoing joint replacement were obtained (with IRB approval) by collagenase digestion. After passages (P6–9) to obtain fibroblast-like synoviocytes, cells were incubated under basal conditions (DMEM+0.5% FBS as control) and then with the addition of cytokines individually or in combination for 3d.

Individual Cytokine Treatment. Individual cytokines were applied over a range of concentrations: IL-1β (0.1, 1, 10 ng/ml), IL-17 (0.1, 10 ng/ml), TGF-β1 (0.1, 1 ng/ml), and TNF-α (1, 100 ng/ml). Combination Cytokine Treatment. Based on dose-responses of individual cytokines, combinations of cytokines were applied at low or high concentrations to assess sub-maximal and maximal stimulation responses: IL-1β (0.1, 1 ng/ml), IL-17 (0.1, 10 ng/ml), TGF-β1 (0.1, 1 ng/ml), and TNF-α (1, 100 ng/ml). Lubricant Secretion Analysis. Secretion rates (rHA, rPRG4) were assessed by analyzing conditioned medium for HA and for PRG4 by ELISA, using HA-binding protein and mAb GW4.23 (gift from Dr. Klaus Kuehntmann), respectively. Rates were normalized to cell number (based on DNA) and culture duration. Statistical Analysis. Data were log transformed to improve normality. For individual cytokines, effects were analyzed by 1-way ANOVA and Dunnett’s post-hoc test. For combinations of cytokines, a 4-way ANOVA (factors: IL-1β, IL-17+IL-32, TGF-β1, and TNF-α) was used to assess individual and interactive (i.e., non-additive) effects of cytokines.

Results: Individual Cytokine Treatment. rHA was affected markedly by IL-1β, and to a lesser extent by TNF-α and TGF-β1 (p<0.05), with ctrl rHA of 0.67±0.15 μg/(106 cell*day) increased to 22x, 4.2x, and 2.8x, respectively (Fig 1A). rPRG4 was affected only by TGF-β1 (p<0.05), with ctrl rPRG4 of 0.06±0.08 μg/(106 cell*day) increased to ~60x (Fig 1B). Combination Cytokine Treatment. Non-additive (i.e., synergistic) upregulation of rHA occurred when TNF-α was present with either IL-1β or the combination of IL-17 and IL-32 (p<0.001), increasing rHA to ~70x, markedly higher than the predicted additive effects (1x and 6.6x) of individual cytokines (Fig 2A). rPRG4 was not further increased by the combination of TNF-α, IL-1β, IL-17, and IL-32. Synergistic effects on rHA were detected with combinations of cytokines at high concentrations but not low concentrations. There was a trend for upregulated rPRG4 in combinations including TGF-β1, increasing from 0.29±0.19 μg/(106 cell*day) to ~3.5x. However, at these cytokine doses, these effects on rPRG4 were not statistically significant (Fig 2B).

Discussion: This is the first report of synergistic upregulation of synoviocyte HA secretion by TNF-α with the combination of IL-17 and IL-32, as well as with IL-1β. The effects of individually applied cytokines are consistent with, and extend, previous studies on the regulation of secretion of HA and PRG4. The synergistic role for IL-17 and/or IL-32 suggests the involvement of T cells and macrophages, respectively, in the regulation of HA. The differential regulation of HA and PRG4 by IL-1β and TGF-β1, and the synergistic regulation of HA by other cytokines may allow modulation of the concentration of individual lubricants to desired levels in bioreactors. The marked upregulation in HA secretion due to synergistic effects of cytokines present in inflammation may explain marked SF volume increases in some injured and diseased joints, with only moderate decreases in SF HA concentration. The synergistic effect of TNF-α with multiple cytokines suggests that therapies targeting TNF-α may have potent effects, i.e., much more than those expected based on blockade of the individual effects of TNF-α.

Figure 1. Effects of individual cytokines at a range of concentrations on HA and PRG4 secretion.

Figure 2. Effects of combinations of cytokines (high concentrations) on HA and PRG4 secretion.