INTRODUCTION: In early osteoarthritis (OA), cartilage matrix degradation is marked by loss of proteoglycans and cleavage of type II collagen (1). Inflammatory cytokines such as IL-1 and TNFα can induce cells in the joint to produce matrix metalloproteinases (MMPs) that in turn are responsible for degradation of the cartilage matrix (1). Additionally, there is a redundancy of action between IL-1β and TNFα (2). This redundancy suggests a successful OA treatment would need to be able to inhibit both of these cytokines.

One such inhibitor for IL-1 is IL-1ra and one inhibitor for TNFα is sTNF-RI. Autologous protein solution (APS) is rich in IL-1ra and sTNF-RI and can be prepared in less than 30 minutes from whole blood (3). The purpose of this study is to demonstrate that APS can inhibit MMP-13 production from chondrocytes simultaneously stimulated with IL-1β and TNFα.

METHODS:

Cell Culture Functional Assay: Human knee articular chondrocytes (Clonetics NHAC, Lonza Inc., Walkersville, MD) were seeded at a density of 20,000 cells/cm² in growth medium (CGM bullet kit and Chondrocyte basal medium, Lonza Inc., Walkersville, MD) in a 12-well plate and incubated at 37°C in 5% CO₂ in air for 24 hours (n=3). Two hours before the assay began, the medium was replaced with serum-free medium. Recombinant IL-1β (5 ng/ml, Sigma, St. Louis, MO) and rhTNFα (100 ng/ml, Prospec Protein Specialists, Rehovot, Israel) were added to the indicated wells. The test wells were pre-incubated with rhIL-1ra (500 ng/ml, Prospec Protein Specialists, Rehovot, Israel) or sTNF-RI (3 μg/ml, R&D Systems, Minneapolis, MN) for two hours before addition of IL-1β and TNFα. Following incubation for 24 hours, the supernatants were removed and frozen at -50°C. Cells were trypsinized and counted in each well. The supernatants were assayed for MMP-13 (Human Quantikine kit, R&D Systems, Minneapolis, MN) by ELISA.

Autologous Solution in Functional Assay: APS was created from 10 human volunteers (IRB HBD-001) as described previously (3). Briefly, platelet-rich plasma (PRP) was created from 60 ml of blood using the GPS III® System (Biomet Biologics, Warsaw, IN). Six milliliters of PRP were collected and added to a modified plasma concentration device (Plasmax® separator, Biomet Biologics, Warsaw, IN). The plasma concentration device was centrifuged for 2 minutes at 2,000 rpm to separate the serum fraction. Anti-inflammatory and inflammatory cytokine content of the APS was analyzed by ELISAs (Human Quantikine kits, R&D Systems, Minneapolis, MN).

Human knee articular chondrocytes were cultured as above in 12-well plates (n=3). The medium was replaced with serum-free medium and APS (0.5 ml) was added to the wells above the cells in a transwell insert (0.4 μm pores, Corning Inc., Corning, NY) two hours before addition of IL-1β (5 ng/ml) or TNFα (100 ng/ml). Following incubation for 24 hours, the supernatants were removed and frozen at -50°C. Cells were trypsinized and counted in each well. The supernatants were assayed for MMP-13 by ELISA.

Data Analysis: MMP-13 concentration was normalized to cell number. Data are presented as mean ± standard deviation. Differences between groups was determined with a Student’s t-test (α=0.05).

RESULTS: Recombinant IL-1β and TNFα together significantly increased MMP-13 production from chondrocytes compared to IL-1β (p=0.04) and TNFα individually (p=0.02) (Fig.1). Recombinant IL-1ra was able to block 91% of IL-1β stimulated MMP-13 production, but only 71% of IL-1β and TNFα stimulated MMP-13 production. Recombinant sTNF-RI was able to block 12% of TNFα stimulated MMP-13 production, and 27% of IL-1β and TNFα stimulated MMP-13 production. However, the best blocking for cells stimulated simultaneously with IL-1β and TNFα was when both IL-1ra and sTNF-RI were used together (blocking 82%) (Fig. 1). These results suggest that in order to minimize the MMP-13 production by IL-1β and TNFα, inhibitors of both cytokines will be required.

APS from 10 human volunteers inhibited MMP-13 production from chondrocytes stimulated with IL-1β and TNFα (Fig. 2). On average, the anti-inflammatory cytokines in the APS contained 51.6 ± 18.3 ng/ml IL-1ra and 2.9 ± 0.5 ng/ml sTNF-RI while the inflammatory cytokines were significantly lower (< 10 pg/ml IL-1β and TNFα). While significant donor-to-donor variability was seen, every sample was capable of MMP-13 reduction compared to the positive control.

DISCUSSION: Early osteoarthritis has been characterized by a degradation of cartilage matrix instigated by inflammatory cytokines (1). In this study, the most efficient inhibition of MMP-13 produced by IL-1β and TNFα stimulation of chondrocytes was seen when both IL-1ra and sTNF-RI were used together. APS contains high concentrations of IL-1ra and sTNF-RI, without expressing large concentrations of IL-1β and TNFα. APS was able to decrease the MMP-13 production from chondrocytes stimulated with the inflammatory cytokines.

In this study, IL-1ra was a more effective inhibitor than sTNF-RI. Previous studies have stated that the required dose of IL-1ra needed to block the activity of IL-1 ranges from 10 to 1000 times greater than IL-1 (4). Previous studies have also suggested a required dose of sTNF-RI needed to be 33-times greater than TNFα to block 50% of TNFα cytotoxicity, and the sTNF-RI dose needed to be 333-times greater than TNFα for >80% prevention of cytotoxicity (5). The APS contained more than 9000X IL-1ra over IL-1β and more than 400X sTNF-RI over TNFα. These anti-inflammatory cytokines are accompanied by platelet-derived anabolic cytokines also present in APS solution (data not shown).

This study provides a platform to test antagonist formulations that are under development. In addition, the results emphasize the role IL-1 and TNFα play in the breakdown of cartilage matrix seen in osteoarthritis. APS may be a potential candidate to provide autologous anti-inflammatory cytokines to neutralize the inflammatory effects of IL-1 and TNFα.

REFERENCES:
2. Abbas et al., Cytokines, Cellular and Molecular Immunology, 2003.
3. Woodell-May, J. et al., 2009; ORS.