LIPOPOLYSACCHARIDE (LPS) HAS DIFFERENT EFFECTS ON PROTEOGLYCAN SYNTHESIS AND DEGRADATION BY CELLS FROM THE ANNULUS FIBROSUS, NUCLEUS PULPOSUS AND ARTICULAR CARTILAGE

* Aota, Y; * An, H; * Imai, Y; * * * * * * Thonar, E; * * * * * Pichika, R; ** Lenz, M; + * * * Masuda, K
+ * Department of Orthopedic Surgery, Rush Medical College, 1653 West Congress Parkway, Chicago, IL 60612

INTRODUCTION: Lipopolysaccharide (LPS), a major component of the outer membrane of gram-negative bacteria, has a deleterious effect on the metabolism of chondrocytes [1]. Exposure of the cells to LPS causes a rapid decrease in the content of aggrecan, the major cartilage proteoglycan (PG). This is the result of both a down-regulation of aggrecan synthesis and an increase in aggrecan degradation [2]. The mechanisms through which LPS exerts its effects on chondrocytes are not yet fully characterized but it appears binding of LPS to the CD14 receptor is involved [2]. LPS markedly stimulated cytokine production by a mixture of nucleus pulposus (NP) and annulus fibrosus (AF) cells [3]. It also has been shown that NP and AF cells respond differently to chemical stimulants, possibly a reflection of individual aspects of their phenotype [4].

The purpose of this study was to test the hypothesis that LPS has different effects on PG synthesis and degradation in cells expressing different levels of the “chondrocytic phenotype”, i.e. NP, outer AF and articular chondrocytes.

METHODS: Cells were harvested from the bovine steer intervertebral disc. The disc was divided into the NP and the inner and outer layers of the AF. However, only the NP and outer AF were used in the experiments reported here. Bovine articular cartilage (AC) was obtained from the metacarpophalangeal joint of the same animal. After enzymatic digestion, cells were resuspended in 1.2% low viscosity sterile alginate beads at 2 million cells/ml [5]. The resulting alginate beads containing different cell populations were cultured in complete medium (DMEM/F12 supplemented with 10% FBS and 25 µg/ml ascorbic acid), changed daily. After 7 days in culture, the cells were cultured for 3 additional days in the absence (Control) or presence of LPS (Sigma-Aldrich) at 0.0005 mg to 5 µg/ml. At the end of the culture period, the beads were dissolved and the cell-associated matrix (CM) and further removed matrix (FRM) separated [6].

PG synthesis: The rate of PG synthesis was measured during the last 4 hours of culture on day 14 using uptake of $^{35}$S-sulfate into PGs, followed by a rapid filtration assay [7].

PG content: Total PG content was assessed using the DMMB method [5].

PG degradation: The rate of PG degradation was measured as previously described [6]. On day 7 of culture, 300 beads were incubated in the presence of $^{35}$S-sulfate at 20 µCi/ml for 16 hours. After washing to remove the unincorporated radioisotope, the beads were cultured for 1, 3 or 5 more days in complete medium with or without LPS at 0.05 µg/ml, changed daily. At each time point, the content of $^{35}$S-PGs in the CM, FRM and medium was measured. The rate of degradation was expressed as a percentage of $^{35}$S-PG remaining in the CM [6].

Statistical analysis: Statistical analysis was performed using one-way ANOVA for PG synthesis, with the Fisher’s PLSD test as a post hoc test, and the unpaired T test for the rate of $^{35}$S-PG degradation. A level of p < 0.01 was used to determine statistical significance.

RESULTS: LPS strongly suppressed PG synthesis by AC cells in a dose-dependent manner (p < 0.0001, Fig. 1). A similar, but less pronounced, suppressive effect was also noted in the case of NP cells (p < 0.001 at 0.05 ng, 0.0001 at 0.5-5 ng) but not of outer AF cells (Fig.1). The total PG content (DMMB) was significantly decreased in a dose-dependent manner in AC and NP beads treated with LPS, but not in outer AF beads treated with this agent (data not shown).

At each time point, treatment with LPS significantly increased the rate of $^{35}$S-PG loss from AC (data not shown in Fig. 2) and NP beads (p=0.001) but it had only a minimal effect in the case of the $^{35}$S-PG present in outer AF beads (Fig. 2).

DISCUSSION: LPS was very effective in altering the rates of synthesis as well as degradation of PGs in containing NP and AC cells, but it was ineffective in altering the metabolism of PGs by AF cells. It is tempting to postulate that the differences in the response of the two types of intervertebral disc cells are related to differences in the content of CD14 receptor molecules present on the surfaces of the NP and outer AF cells. This hypothesis is being investigated in our laboratory.

REFERENCES:

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** Dept. of Biochemistry, Rush Medical College, Chicago, IL
*** Dept. of Internal Medicine, Rush Medical College, Chicago, IL