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Interleukin-1β affects the phospholipid biosynthesis of fibroblast-like synoviocytes from human osteoarthritic knee joints

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SUMMARY

Objective: Phospholipids (PLs), together with hyaluronan and lubricin, are involved in boundary lubrication within human articular joints. Levels of lubricants in synovial fluid (SF) have been found to be associated with the health status of the joint. However, the biosynthesis and release of PLs within human joints remains poorly understood. This study contributes to our understanding of the effects of cytokines on the biosynthesis of PLs using cultured fibroblast-like synoviocytes (FLS) from human osteoarthritic knee joints.

Methods: Cultured FLS were stimulated with IL-1β, TNFα, IL-6, or inhibitors of cell signaling pathways such as QNZ, SB203580 and SP600125 in the presence of stable isotope-labeled precursors of PLs. Lipids were extracted and quantified using electrospray ionization tandem mass spectrometry (ESI-MS/MS).

Results: Our analyses provide for the first time a detailed overview of PL species being synthesized by FLS. IL-1β increased the biosynthesis of both phosphatidylethanolamine (PE) and PE-based plasmalogens. We show here that the NF-κB, p38 MAPK and JNK signaling pathways are all involved in IL-1β-induced PL biosynthesis. IL-6 had no impact on PLs, whereas TNFα increased the biosynthesis of all PL classes.

Conclusion: The biosynthesis of various PLs is controlled by IL-1β and TNFα. Our detailed PL species analysis revealed that FLS can partly contribute to the elevated PL levels found in human osteoarthritis (OA) SF. IL-1β in particular stimulates PE and PE-based plasmalogens which can act as cell-protective antioxidants. These results suggest that during OA progression, FLS undergo alterations in their PL composition to adapt to the new diseased environment.

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Introduction

Phospholipids (PLs) together with hyaluronan and lubricin have been reported to provide boundary lubrication within human articular joints[1–4]. Recent studies have proposed that lubricin interacts with hyaluronan laden with PLs, which enables sliding due to hydration lubrication[3,5,6]. We previously reported that synovial fluid (SF) from patients with osteoarthritis (OA) and rheumatoid arthritis (RA) display higher levels of a large variety of PL species compared to controls[7,8], whereas the concentrations of hyaluronan and lubricin were reduced in these diseases[9]. The elevated levels of phosphatidylcholines (PC) seem to compensate for the decreased levels of hyaluronan and lubricin in providing proper lubrication so that cartilage surfaces are protected against wear. Moreover, the increased content of phosphatidylethanolamine (PE)-based plasmalogens (PE P) in OA SF may protect against the destructive effects of reactive oxygen species (ROS) in damaged articular cartilage and membrane PLs[8]. In general, PLs were reported to play a vital part in

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areas such as cell membranes, cell signaling, inflammation, and in anti-oxidative processes[10–13].

The PLs found in SF may originate partly from blood or may indeed also be produced locally by the joint tissue cells. Fibroblast-like synoviocytes (FLS) are major local secretory cells able to synthesize and release PLs which they store in lamellar bodies[14–17]. The PL classes PC, involved in boundary lubrication, as well as the PE class are synthesized *de novo* in endoplasmic reticulum membranes in mammalian cells via the Kennedy pathway[11,18]. FLS can also synthesize and secrete hyaluronan and lubricin, thus supporting the role of these molecules in joint lubrication[19,20]. In addition, the FLS contribute to the local production of cytokines, mediators of inflammation and catabolic enzymes[21].

OA is a complex degenerative joint disease, involving all joint tissues, and is driven by mechanical factors, various inflammatory mediators and growth factors[22]. IL-1β and TNFα number amongst the key cytokines involved in the pathogenesis of OA[23,24]. IL-6 is produced in chondrocytes, osteoblasts, FLS, macrophages and adipocytes in response to IL-1β and TNFα. Patients with OA display elevated levels of these cytokines in SF and serum[25–28]. Remarkably, the expression of IL-R1 receptors and TNF-R2 is also increased in FLS and chondrocytes isolated from patients with OA[24]. IL-1β and TNFα self-stimulate their own production and induce the expression of IL-6, IL-8, CCL5, and COX-2 in FLS and chondrocytes[23,24]. They also stop chondrocytes from producing extracellular matrix components such as collagen type II and aggrecan, but stimulate these cells to produce catabolic enzymes like MMP-1, -3, and -13 and ADAMTS[24,29]. IL-6 is a cytokine which strongly enhances the inflammatory response, although some of its effects might be anti-inflammatory.

Some cytokines have already been found to affect the biosynthesis of articular joint lubricants. As such, the expression of lubricin by synoviocytes and chondrocytes was reported to be inhibited by IL-1 and TNFα, whereas TGF-β1 upregulates lubricin synthesis[30,31]. In contrast, hyaluronan production by human FLS was found to be stimulated by IL-1, but inhibited by TGF-β1[32]. Using OA FLS, Angel *et al.*[33] reported that IL-1β induces the breakdown of PC and PE through the activation of phospholipase A2. Kronqvist *et al.*[34] investigated the effect of IL-1β on the synthesis and release of cholesterol, PC, and sphingomyelin (SM) from normal human skin fibroblasts. Long-term exposure to IL-1β markedly increased the rate of cholesterol esterification, reduced SM synthesis, but barely affected the PC synthesis, whereas the efflux of these lipids to lipid-free apolipoprotein was enhanced. However, data on individual PL species is lacking since this was not addressed by the methodology. Moreover, the impact of IL-1, IL-6 and TNFα on the biosynthesis of PLs from human FLS including individual classes and species is unknown.

Given the association between cytokines and lubricant production by FLS during OA, our intention was to determine the individual effects of IL-1, TNFα and IL-6 on the synthesis of different PL classes and species in human OA FLS, and further elucidate the cell transduction mechanisms involved. We found that the PL biosynthesis of OA FLS is regulated in a sophisticated manner by the cytokines tested. These data may aid the exploration of the mechanisms responsible for modifying PL metabolism within articular joints during the progression of OA.

Materials and methods

Fibroblast-like synoviocytes

FLS were obtained from OA patients undergoing total knee replacement surgery as described elsewhere[35]. The study was approved by the local ethics committee of the Justus Liebig University Giessen, and all patients provided written consent to donate samples for research. The effect of recombinant human cytokines on FLS was tested with cells derived from patients who fulfilled the following inclusion criteria: diagnosed OA, both genders, age 50–85 years, and BMI 20–35. FLS were excluded from the study from patients with (1) other joint disease such as RA, gout, or trauma, (2) knee joint surgery during the preceding 6 months, (3) severe diseases like HIV infection, tumor(s) near to the joint, severe liver and kidney diseases, drug abuse and (4) intake of immunosuppressive drugs, corticosteroids or hyaluronan during the preceding 6 months. Due to the exclusion criteria, samples of about 40% of all patients were not used for this study.

Cell culture

FLS were cultured in a humidified 10% CO_2 atmosphere at 37°C using DMEM medium supplemented with 1.0 g/l glucose, 584 mg/l L-glutamine, 10% fetal bovine serum (FBS), 10 mM HEPES buffer, 10 U/ml penicillin and 0.1 mg/ml streptomycin. The experiments were performed with cells harvested at the end of passage 4 or 5. Routine tests for mycoplasma contamination using a PCR Mycoplasma Kit (PromoCell) were negative. The purity of FLS was determined at the end of passage 4–5 with a BD FACSCANTO II flow cytometer (Becton Dickinson) after trypsinization. Using APC anti-human CD90 antibodies (clone 5E10, BioLegend), more than 90% of the cells stained positively for the fibroblast-specific antigen CD90, whereas staining with PE anti-human CD45 antibodies (clone 2D1, BioLegend) confirmed that the cells were CD45-negative.

Biosynthesis model

For analysis of PL biosynthesis, cells of passage 4 or 5 were seeded into 6-well plates at a density of 80,000 FLS per well. Cells were grown until 100% confluency and then starved for 24 h in serine- and choline-depleted, phenol-free DMEM medium (PAN Biotech) containing 5% lipoprotein deficient serum (LPDS, generously supplied by Dr. A. Sigruener), 10 mM HEPES buffer, 10 U/ml penicillin, 0.1 mg/ml streptomycin and 4 mg/l folic acid. Afterwards, media were changed and the cells were labeled with 225 μg/ml of [D9]-choline and 25 μg/ml of [D4]-ethanolamine (Cambridge Isotope Laboratories) for 8–36 h. Cells were washed twice with 1× PBS and lysed with 0.2% of sodium dodecyl sulfate (SDS, Sigma). Wells were washed with double-distilled water and combined extracts were treated with ultrasound for 6 s at 40–50% power (Sonoplus, Bandelin electronic GmbH). The protein concentrations of cellular lysates were quantified using the Pierce™ BCA Protein Assay Kit (Thermo Fisher). Our preliminary tests revealed that FLS maintained a stable metabolism as indicated by their unaltered expression of the reference genes B2M, βactin, and GAPDH (QuantiTect® Primer Assays, Qiagen), their constant mitochondrial activity (Cell Titer 96®, Promega), and their high cell viability (>90%, trypan blue exclusion test, Sigma) and negligible caspase 3/7 activity (Caspase-Glo® 3/7 Assay, Promega).

Effect of cytokines and agents on the biosynthesis of PL

FLS from six patients were labeled with 225 μg/ml [D9]-choline and 25 μg/ml [D4]-ethanolamine for 16 h and simultaneously treated either with 10 ng/ml IL-1β (R&D Systems), IL-6 (Life Technologies), or 100 ng/ml TNFα (Peprotech). To elucidate the signal transduction mechanism of IL-1β used for PL biosynthesis, FLS from five patients were first pre-incubated for 30 min with a 10 μM solution of a cell-permeable inhibitor of signal transduction (Selleckchem), namely the NF-κB activation inhibitor QNZ, the p38 MAPK inhibitor SB203580, or the broad-spectrum JNK inhibitor SP600125. Cells were then stimulated with 10 ng/ml IL-1β for 16 h in the continued presence of the inhibitors.

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