DIFFERENTIAL EFFECTS OF IL-8, LIF AND IL-11 ON THE TNF-ALPHA-INDUCED PGE2 RELEASE AND ON SIGNALING PATHWAYS IN HUMAN OA SYNOVIAL FIBROBLASTS

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INTRODUCTION

Studies on the contribution of cytokines to the pathogenesis of osteoarthritis (OA) have focused mainly on two proinflammatory cytokines, IL-1ß and TNF-α. In OA, the specific causative of the pathological process has not yet been identified, however episodic inflammation at the clinical stage of the disease plays a pivotal role. TNF-α appears to be a meaningful cytokine involved during this process, thus representing an important therapeutic target. The mechanisms by which the inflammatory response is maintained, however, remain poorly understood.

Amplification and perpetuation of the degradative process in articular joint tissue is dependent on a complex interplay between ambient cytokines. The net effect of proinflammatory cytokines depends not only on absolute levels but on their interaction at the intracellular signaling cascade. Other cytokines having inflammatory properties could contribute to this pathological process; therefore acting on specific regulatory agents may represent a natural way to prevent the consequences of inflammation in OA. In the present study, we investigated the effects of three prominent cytokines that are classified as inflammatory agents, these being IL-8, LIF and IL-11. They are upregulated by TNF-α, and may potentially amplify and propagate the biological response of this proinflammatory cytokine.

More specifically, we investigated on human OA synovial fibroblasts the effects of IL-8, LIF and IL-11 on TNF-α biological activity in terms of PGE_2 production, and their impact on points of potential intervention in the cell signaling cascades initiated by TNF-α. This included effects on the TNF-receptors, TNF-soluble receptors, cytoplasmic phospholipase A2 (cPLA2), cyclooxygenase-2 (COX-2), and the transcription factors NF-κB and C/EBP.

MATERIALS AND METHODS

Synovial membrane specimens were obtained from OA patients undergoing total knee joint replacement. Synovial fibroblasts were released from the synovial membrane by sequential enzymatic digestion at 37 C, and cells were seeded at high density. First passage cells were used, and analyses were conducted to confirm that no monocytes/macrophages were present in the fibroblast preparations.

OA synovial fibroblasts were incubated in the presence or absence of increasing concentrations of IL-8 (0.05-5 ng/ml), LIF (0.5-50 ng/ml and IL-11 (0.5-25 ng/ml) for 24h prior to the addition of TNF-α (5 ng/ml) for an additional 48h. Culture media were recovered, and PGE_2, TNF-sR55 and TNF-sR75 were determined using specific ELISAs. Following this, radioreceptor binding analysis was performed on the cells.

The effects of IL-8, LIF and IL-11 on TNF-α-induced cPLA2 and COX-2 synthesis and cPLA2 activity were also determined following a preincubation of the cytokines for 24h, followed by an incubation of 24h with TNF-α. COX-2 and cPLA2 synthesis were determined following Western blot using specific antibodies. cPLA2 activity was measured with an arachidonic acid substrate, the 1-stearoyl-2 (^{14}C) arachidonyl phosphotidylcholine.

To study the transcription factors, OA synovial fibroblasts were treated for 24h with the cytokines, and incubated for 2h with the TNF-α. Nuclei were isolated, extracted and subjected to electrophorectic mobility gel-retardation and levels of NF-κB and C/EBP determined using the specific sense sequences.

RESULTS

IL-8 increased in a synergistic manner (282% at 5 ng/ml) and LIF in an additive fashion (69% at 50 ng/ml) the TNF-α-induced PGE_2 release, while IL-11 reduced it (83% at 5 ng/ml). IL-8 at 5 ng/ml and LIF at 50 ng/ml alone upregulated (about 30%) the TNF-receptor binding levels, but significantly downregulated the TNF-α-induced ones ($p<0.007$ and $p<0.004$), respectively); IL-11 reduced it by 18% ($p<0.005$) and 51% ($p<0.01$), respectively. All three cytokines downregulated the TNF-soluble R55, while on the TNF-soluble R75, IL-11 decreased it.

IL-8 and LIF, either alone or under TNF-α treatment, increased COX-2 and cPLA2 synthesis levels, and IL-11 inhibited both the basal and the TNF-α-induced ones. Interestingly, on the cPLA2 activity level, induction occurred in a synergistic manner for IL-8, and in an additive fashion for LIF.

NF-κB was upregulated by the cytokines alone; on the TNF-α-induced NF-κB accumulation, IL-8 and LIF had no effect, while IL-11 significantly inhibited it ($p<0.02$). All three cytokines inhibited the TNF-α-induced C/EBP synthesis.

CONCLUSION

These results indicate that IL-8 synergizes and LIF potentiates the TNF-α PGE_2 effect, which appears to be mediated by an increase in cPLA2 activity level.

As a whole, our findings suggest that, on OA human synovial fibroblasts, LIF functions in cooperation whereas IL-8 in parallel with TNF-α on the TNF-α-induced signaling cascade. On the other hand, IL-11 alone had no effect, but in conjunction with TNF-α, this cytokine showed antiinflammatory properties.

This study provides a rational foundation on which to develop therapeutic strategies for the treatment of OA by shedding light on the mechanisms of action of three prominent cytokines at work in articular joint tissues during the inflammation process.

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