CHARACTERISATION OF THE METABOLISM OF OSTEOARTHRITIC CHONDROCYTES CULTURED IN ALGINATE BEADS

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AIM OF STUDY: To investigate the production of aggrecan, stromelysin-1 (MMP-3), tissue inhibitor of metalloproteinases (TIMP)-1, interleukin (IL) -6, -8, macrophage inflammatory protein (MIP)-1β, nitric oxide (NO), prostaglandin (PG)E_2 and TGF-β1 by human osteoarthritic (OA) chondrocytes cultured for a long-term in alginate beads. This work gives also new informations on the distribution of these agents in the different compartments of the alginate culture: the cell-associated matrix (CM), that corresponds to the pericellular and territorial matrix in cartilage, and the further-removed matrix (FRM), the equivalent of the interterritorial matrix in this tissue. Finally, we have investigated the long-term effects of interleukin (IL)-1β on the OA chondrocytes metabolism.

METHODS: Human articular OA chondrocytes were cultured for 12 days in alginate beads (n=10), in the absence or the presence of IL-1β 10^{-10} M. The culture supernatant was changed every three days. The production of aggrecan, MMP-3, TIMP-1, IL-6, IL-8, MIP-1β and TGF-β1 were investigated in the different matrix compartments and in the culture supernatant by specific ELISA. The PGE_2 released into the supernatant was also quantified by RIA and ·NO by a spectrophotometric assay based upon the GRIESS reaction.

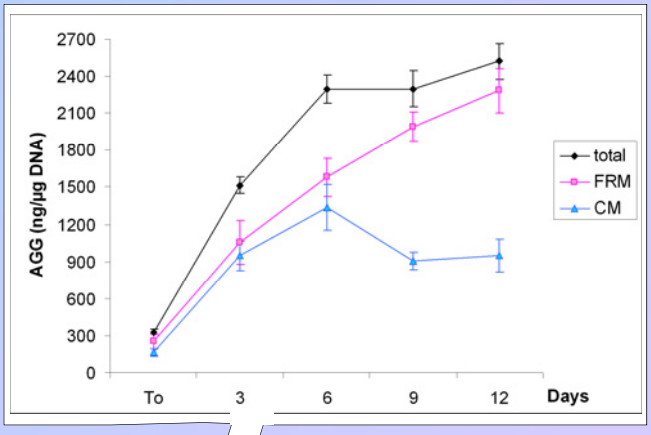
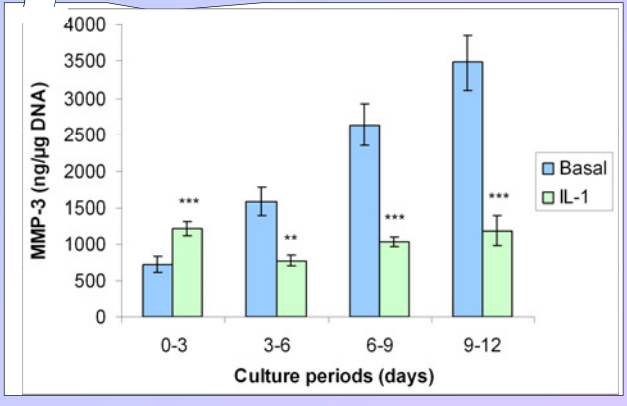


Table I: Distribution of cytokines, MMP-3, TIMP-1 and aggrecan in the alginate culture compartments (percentage of total production)

		CM	FRM	Supernatant
Aggrecan	- IL-1β	24.70	73.51	1.79
	+ IL-1β	22.08	71.62	6.31
MMP-3	- IL-1β	0.11	4.56	95.34
	+ IL-1β	0.15	3.82	96.04
TIMP-1	- IL-1β	0.80	5.37	93.83
	+ IL-1β	1.08	7.92	91.00
IL-6	- IL-1β	0.17	4.44	95.39
	+ IL-1β	0.12	3.94	94.93
IL-8	- IL-1β	4.96	16.18	78.85
	+ IL-1β	3.81	23.75	72.45
MIP-1β	- IL-1β	1.07	2.67	96.26
	+ IL-1β	0.53	2.17	97.31
TGF-β1	- IL-1β	11.27	34.42	54.32
	+ IL-1β	13.59	28.38	58.02



RESULTS: As shown in the table I, cytokines, metalloproteinases and aggrecan are differently distributed in the different compartments of the alginate culture. Aggrecan was primarily found in the CM and FRM, whereas PGE_2, ·NO, MMP-3 and TIMP-1 were almost exclusively found in the supernatant. In contrast to other cytokines (MIP-1β and IL-6), IL-8 and TGF-β1 were accumulated in the extracellular matrix. At the end of the 12 days of incubation, IL-1β strongly stimulated IL-6, IL-8, MIP-1β, PGE_2 and ·NO production but dramatically inhibited aggrecan, TIMP-1 and TGF-β1 synthesis. After 6 days of culture, AGG amount increased in the FRM and decreased in the CM whereas total remained stable suggesting that newly synthetized AGG migrate to the FRM.

The IL-1β-induced MMP-3 production showed a particular profile. Indeed, the chronic administration of IL-1β stimulated MMP-3 during the first three days of culture. Nevertheless, after the second treatment, this effect disappeared.

CONCLUSION: Alginate beads is a suitable model for studying cartilage matrix formation. IL-8, but not MIP-1β or IL-6, may be accumulated in the extracellular matrix mimicking the IL-8 gradient found in vivo in inflammed joint. Finally, IL-1β deeply dysregulates chondrocytes metabolism, increasing catabolic and pro-inflammatory mediators and decreasing matrix components and growth factor synthesis