**The Variable Effects of Dexamethasone on the Chondrogenic Differentiation of Mesenchymal Stem Cells**

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**INTRODUCTION:** Glucocorticoids regulate in diverse ways a variety of cell-, tissue- and organ-specific biological functions, including differentiation, growth, metabolism and apoptosis. Indeed, their influence on a given cell type can even be diametrically opposed. For example, they can both promote [1, 2] and inhibit [3, 4] the elaboration of a cartilaginous matrix by chondrocytes *in vitro*. Although so, the factors that govern the behaviour of glucocorticoids on a given cell type have not been systematically addressed in a single study. More than a decade ago, dexamethasone (DEX) – a synthetic glucocorticoid – was shown to potentiate the transforming-growth-factor-[β1 (TGF-β1)]-stimulated chondrogenesis of bone-marrow-derived mesenchymal stem cells (MSCs) [5]. And on the basis of this finding, it has since been added on a routine basis to chondrogenic media, irrespective of the tissue origin of the MSCs and the nature of the stimulating growth factor. We suspect that the effects of DEX on the chondrogenic differentiation of MSCs vary according to their tissue origin, their culturing microenvironment and the nature of the stimulating agent. To test this hypothesis, we investigated its effects on the chondrogenesis of bovine MSCs as a function of tissue origin (synovium vs. bone marrow), microenvironment (explants vs. cell aggregates) and the nature of the stimulating growth factor [TGF-β1 vs. bone morphogenetic protein 2 (BMP-2)].

**METHODS:** Synovial tissue and bone marrow cells were obtained respectively from the metacarpal joints and bones of freshly-slaughtered bovine calves. Synovial-explant cultures were established by sandwiching small strips of tissue between two layers of agarose in 24-well plates. Suspensions of synovial cells were prepared from other tissue samples by enzymatic digestion. Bone-marrow cells were collected by scraping. The isolated cells of each tissue type were cultured as monolayers (in DMEM containing 10% FBS) to promote the expansion and expansion of MSCs. After a single passage, the MSCs were suspended at a numerical density of 2 x 10^5/mL. 50-µL aliquots of the suspensions were introduced into the cavities of 12-well plates, which were incubated for 4 hours at 37°C in an atmosphere containing 5% CO₂ to promote the formation of cell-aggregates. Synovial explants and aggregates of synovial and bone-marrow-derived MSCs were then cultured in DMEM containing ITS+Premix (1%), proline (1 mM) and ascorbic acid (25 µg/mL). Chondrogenesis was induced by adding TGF-β1 (10 ng/mL) or BMP-2 (200 ng/mL) to the culture medium, which either lacked or contained DEX (100 nM). After 2 or 4 weeks of culturing, chondrogenic differentiation was gauged by an immunohistological analysis of aggrecan and type-II collagen, and by monitoring the gene-expression levels of these two proteins using the real-time PCR technique [6].

**RESULTS:** In the absence of DEX and a growth factor, neither aggrecan nor type-II collagen was immunohistochemically revealed within either synovial explants or aggregates of isolated synovial or bone-marrow-derived MSCs (Fig. 1A). In the presence of DEX but in the absence of a growth factor, aggrecan (but not type-II collagen) was deposited only within aggregates of bone-marrow-derived MSCs. After stimulation with TGF-β1 in the absence of DEX, immunostaining for aggrecan was intense within synovial explants but weak within aggregates of isolated synovial or bone-marrow-derived MSCs (Fig. 1B). DEX suppressed the TGF-β1-stimulated deposition of aggrecan within synovial explants, but enhanced it within aggregates of isolated synovial or bone-marrow-derived MSCs. Stimulation with TGF-β1 in the absence of DEX led to weak immunostaining for type-II collagen within peripheral portions of synovial explants and within aggregates of synovial MSCs, but no immunoreactivity for this protein was detected within aggregates of bone-marrow-derived MSCs. DEX slightly suppressed the TGF-β1-stimulated deposition of type-II collagen within synovial explants, greatly enhanced the process within aggregates of bone-marrow-derived MSCs, and had no obvious effect on the phenomenon within aggregates of isolated synovial MSCs. After stimulation with BMP-2 in the absence of DEX, aggrecan was deposited within synovial explants as well as within aggregates of isolated synovial or bone-marrow-derived MSCs; type-II collagen was deposited within synovial explants and within aggregates of isolated synovial MSCs, but not within aggregates of isolated bone-marrow-derived MSCs (Fig. 1C). DEX greatly suppressed the BMP-2-stimulated deposition of both aggrecan and type-II collagen within synovial explants, but enhanced the responses within aggregates of isolated synovial or bone-marrow-derived MSCs – greatly in the case of aggrecan for both tissue types and marginally in the case of type-II collagen for bone-marrow-derived MSCs. These immunohistochemical findings for the two proteins (aggrecan and type-II collagen) were confirmed at the gene-expression level.

**DISCUSSION:** Our study reveals that DEX has highly variable effects on the chondrogenic differentiation of MSCs. This glucocorticoid is well known to potentiate the TGF-β1-induced chondrogenesis of bone-marrow-derived MSCs. However, if these cells are stimulated with BMP-2 instead of with TGF-β1, the potentiating effect of DEX on chondrogenesis is less marked. This finding demonstrates that its influence is growth-factor dependent. But irrespective of the nature of the stimulating agent, DEX exerts less influence on aggregates of isolated MSCs that are derived from the synovium than on those that are obtained from bone marrow. This finding demonstrates that the effects of DEX are influenced by the tissue origin of the MSCs. Furthermore, whilst DEX partially enhances the BMP-2-stimulated chondrogenesis of isolated synovial cells, it almost completely inhibits the process in synovial explants. This finding demonstrates that the effects of DEX on the chondrogenesis of MSCs are impacted by their microenvironment. In conclusion, the effects of DEX on the chondrogenic differentiation of MSCs depend upon the tissue origin of the MSCs, the culturing microenvironment and the nature of the stimulating growth factor. DEX is thus not indispensable for the chondrogenesis of MSCs, and can even inhibit the process.


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